



Molecular Mechanism of Coronaviruses (COVID-19) and Diagnostic Approaches: A Systematic Review

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ABSTRACT

An acute respiratory disease is rampantly spreading in population worldwide caused by a novel coronavirus (SARS-CoV-2, also known as COVID-19). The COVID-19 is a major source of disaster in the 21st century. It has spread throughout China and is received as a pandemic worldwide. Till date (18th May, 2020), a total of 4,827,272 patients are infected and more than 3,17,174 confirmed deaths have been reported with 6.57% fatality rate. Several research investigations have identified that COVID-19 belongs to β -coronavirus family and has a highly identical genomic structure to bat coronavirus. The novel coronavirus uses the same receptor, ACE-2 (angiotensin-converting enzyme 2) as that for SARS-CoV, and mainly spreads through the respiratory tract. As per WHO, symptoms include shortness of breath especially in the lower respiratory tract, sore, throat, cough, headaches and fever. However, the specific drugs required to prevent/treat an attack is a major need at this current point of time. In this regard, we conducted a systematic review on coronavirus to cover the molecular mechanism of viral entry and replication, which provides the basis of future management of COVID-19.

Keywords: Novel coronavirus COVID-19, ACE-2, glycosylated protein and endocytosis.

1 Introduction

Coronaviruses is a constituent of the subfamily Orthocoronavirinae (subgenus *arbecovirus*), which causes diseases in mammals and birds (1). Coronavirus word is derived from Latin word corona, means “crown”. Other members of this family are *Coronaviridae*, *Nidovirales*, and realm *Riboviria* (2). In human they cause respiratory tract infections that can be mild, but COVID-19, SARS and MERS can be lethal, if immune system is not working strongly (3). Symptoms of COVID-19 are shortness of breath, sore throat, cough, headaches, and fever. According to the Centers for Disease Control and Prevention (CDC), coronavirus occasionally cause complications in the lower respiratory tract. The infected droplets can spread up to 1-2 meter and deposit on surfaces. The virus can remain live on hard surfaces for days in favourable conditions. Infection can be spread by inhalation of infected droplets (aerosolization/fecooral route) of virus or touching contaminated surfaces. The virus is also present in the stool and contaminated water (68). Virus can be destroyed in a minute by common disinfectants like sodium hypochlorite, hydrogen peroxide, 70% ethanol or even methanol etc(4). Therefore, washing your hands frequently with soap or detergents, especially after contact with infected person or environment is advised.

In 1930, Coronavirus were first discovered in avian with an acute respiratory infection in chickens (5). In 1960 human Coronaviruses were discovered as common cold virus and later named as human coronavirus 229E and human coronavirus OC43 (6). Other human coronaviruses have been involved in serious respiratory tract infections including SARS-CoV in 2003, HCoV NL63 in 2004, HKU1 in 2005, MERS-CoV in 2012, and COVID-19 in 2019. Noble Coronavirus (COVID-19) first appeared in China, Wuhan and officially declared in December 2019, initially named 2019-nCoV by World Health Organization (WHO), January 2020. Now, COVID-19 has become a pandemic worldwide. WHO officially declared the COVID-19 epidemic as a public health emergency of international concern (7). Chinese scientists isolated a COVID-19 from a patient on 7th January 2020 and came out with the genome sequencing of the COVID-19 (8). The Wuhan strain has been identified as a new strain of β -coronavirus (Refer figure 3) and has 70% genetic similarity with SARS-CoV. The virus has a 96% similarity to a bat coronavirus, so it is widely suspected to have been originated from the bat as well (8, 9, 10).

As of 18th May 2020, a total 4,827,272 patients have been infected, while roughly 1,68,532 have recovered and 3,17,174 have been confirmed dead in the coronavirus pandemic (70). The COVID-19 epidemic has reached nearly 213 countries (11). The pandemic has resulted in travel restrictions and nationwide lockdowns in several countries. Table 1 shows the most infected countries with COVID-19.

Table 1: Coronavirus cases and deaths country wise (Data as on 18th May 2020).

S. No	Country	Confirmed Cases	Deaths	Fatality rate
	Worldwide	4,827, 272	3,17,174	6.57%
	USA	1,528,179	90,988	5.95%
	Russia	2,90,678	2,722	1.26%
	Spain	2,77,719	27,650	9.95%
	UK	2,43,695	34,636	14.21%
	Brazil	2,42,313	16,192	6.68%
	Italy	2,25,435	31,908	14.15%
	France	1,79,569	28,108	1.56%
	Germany	1,76,788	8,052	4.55%
	Turkey	1,49,435	4,140	2.77%
	Iran	1,22,492	7,057	5.76%
	India	96,169	3,029	3.14%

The COVID-19 caused by the coronavirus is a β -coronavirus with enveloped non-segmented positive-sense RNA (12). They are classified into three groups, based on antigenic spike (S), membrane (M) and nucleocapsid (N) proteins. Coronaviruses (CoV) are divided into four genera, including alpha (α), beta (β), gamma (γ) and delta (δ). α and β coronaviruses (Refer figure 3) are able to infect mammals, while γ and δ coronavirus infect only avian species. In humans, from commonly known seven coronaviruses which cause disease, four including 229E, OC43, NL63 and HKU1 have mild effect. Three of the coronaviruses can be lethal for humans as SARS (severe acute respiratory syndrome) which emerged in late 2002 and disappeared by 2004; MERS (Middle East respiratory syndrome), which emerged in 2012 and remains in circulation in camels; and COVID-19, which emerged in December 2019 from China and a global effort is under way to contain its spread (7).

2 Genomic Organization of Coronaviruses

Coronaviruses are enveloped virus consisting of a lipid bilayer membrane, enveloped and spike structural proteins (13), with a positive-sense single-stranded RNA genome and a nucleocapsid of helical

structure (2,14). This structure helps to protect the virus when it is outside the host cell (14) as shown in Figure 1(a) (69). It also has a shorter spike-like surface protein called hemagglutinin esterase (HE) (10). Coronaviruses consist one of the largest genome size of 27 to 34kilo bases in the RNA virus family (15). Its genomes have a 5' methylated cap and a 3' polyadenylated tail. Coronaviruses have a diameter of about 120nm (9).

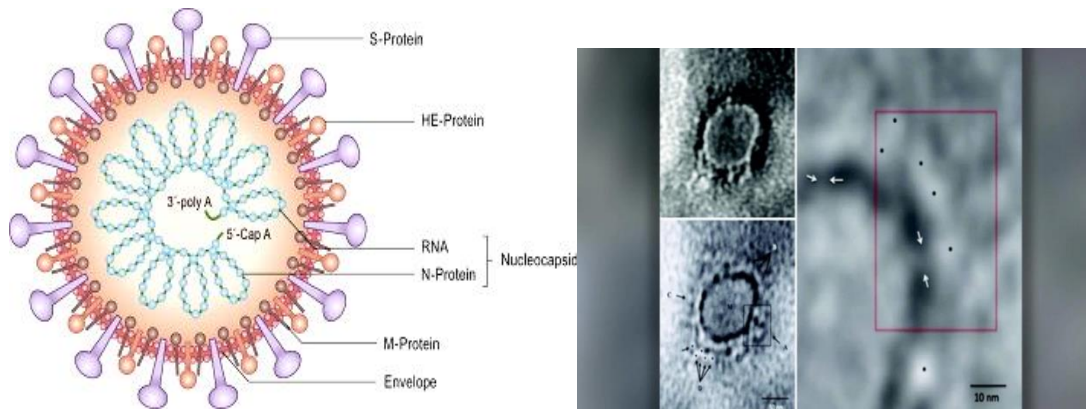


Figure 1. (a). Structure of coronavirus (69) **(b).** Transmission electron microscopy imaging of COVID-19 (71)

Figure 1(b) shows, the first image of COVID-19 from Indian Council of Medical Research (ICMR, India) captured through Transmission Electron Microscopy (TEM) (71). The sample was taken from the throat swab of the first laboratory confirmed patient on 30th January 2020 in Kerala, India. Image shows the round shape of the virus as well as projections or stalks jutting out from the surface of the novel coronavirus particles.

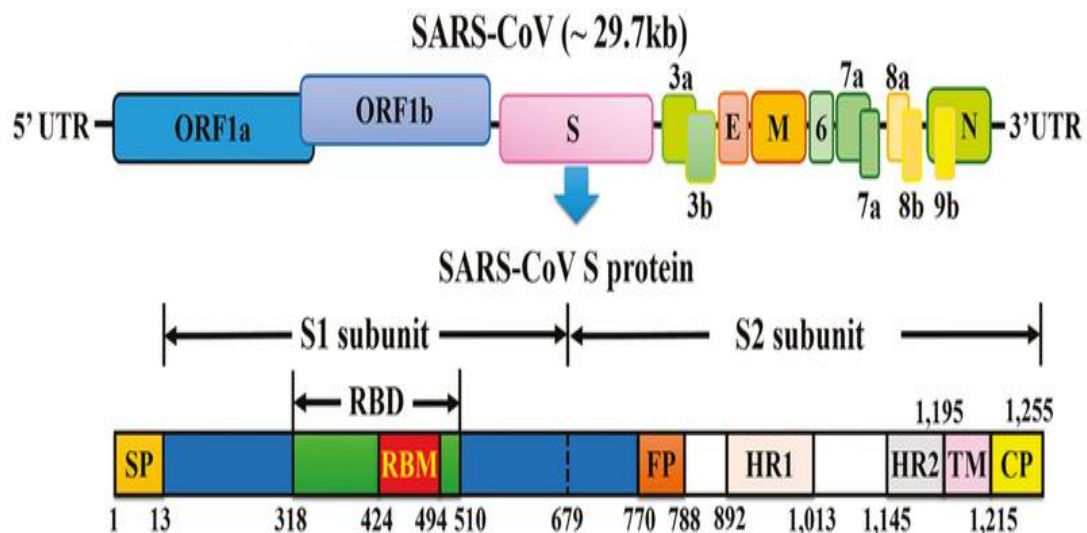


Figure 2. Genomic organization of COVID-19 (3).

The genome of coronavirus consists 5'-leader-UTR transcriptase-spike (S)-envelope, (E)-membrane, (M)-nucleocapsid, (N)-3'UTR and poly (A) tail. ORF1a and ORF1b are the open reading frames, cover the two-thirds of the whole genome and produce the two viral replicase proteins (polyproteins) (16). Both ORFs encode total 16 Non-Structural Proteins (NSPs) which are highly conserved in the coronaviruses. These NSPs actively participate to produce replicase transcriptase complex. Transcriptase polyprotein has the ability to self-cleave to form non-structural proteins (3) (Refer figure 2).

The S protein is heavily glycosylated protein, mainly contains the S1 and S2 subunits. These S subunit proteins are processed by host proteases (17). The S1 subunit is responsible for receptor binding and S2 subunit for membrane fusion. Coronavirus consist two subunits within the S1 unit, which are capable of binding to host receptors. One is an amino (N)-terminal domain (NTD) and another is a carboxy (C)-terminal domain (CTD), are protein receptors for COVID-19 and MERS-CoV (18,29). However, S protein in other CoVs as SARS-CoV remains intact on viral particles and only gets cleaved inside endocytic vesicles during viral entry (20,21). The M protein (25-30kDa) is the most abundant structural protein in the virion structure and has 3 trans- membrane domains that give the virion its shape (22). M protein has a small N-terminal glycosylated ectodomain and a much larger C-terminal endodomain that extends 6-8 nm into the viral particle (23).

The E protein with small portion about 8-12 kDa are highly divergent (22,24) (Refer figure 2). E protein of virion has very limited data suggesting that it is a trans-membrane protein with an N-terminal ectodomain, a C-terminal endodomain and has ion channel activity. The ion channel activity in COVID-19 is required for pathogenesis. Studies suggested that E protein of corona virus is not always lethal although depends upon virus type (25). This protein facilitates assembly and release of the virus. N protein present in the nucleocapsid of virus has two domains an N-terminal domain (NTD) and a C-terminal domain (CTD) which helps RNA binding in-vitro (26). N protein also binds NSP3 (Non-Structural Protein3, a key component of the replicase complex) (27,28), and the M protein (29). These proteins help together the viral genome to form the Replicase Transcriptase Complex (RTC), and subsequently package the encapsulated genome into viral particles. Recent studies suggest that the mutation in NSP2 and NSP3 play a role in infectious capability and differentiation mechanism of COVID-19 (30).

3 Action of Mechanism of COVID-19

3.1 Entry of virion into the Host Cell

In early phase of infection, COVID-19 attaches to host cell with the viral spike with (S proteins) glycoprotein Receptor Binding Domain (RBD). As attachment occurs, virus releases protease to cleave host cell and activates the receptor-attached spike protein or S protein receptor interaction. A recent study published in Science showed that COVID-19 S protein has higher affinity to ACE2 (Angiotensin-Converting Enzyme 2) than SARS-CoV S protein (31). ACE2 is anenzymemostly found on the outer cell membranes in the lower respiratory tract, lungs, arteries, heart, kidney, and intestines of humans (32,33,34).Studies showed that β -CoV receptors have a good capacity to bind with the human cells expressing ACE2, dipeptidyl peptidase-4 (DPP4, also known as CD26) or APN (Amino Peptidase N) (35,36). The S1 domain of S protein (Refer figure 2), mediates binding to the cognate host cell receptor; while, the other S2 domain mediates the fusion events, between viral membrane and host cell membrane, that are required for entry of virus into host cells (37).Depending upon host cell protease and membrane nature, cleavage and activation allows thevirus to enterthe host cell byendocytosis. Studies suggest that many coronaviruses utilize peptidases as their cellular receptor but exact mechanism is still unknown (37,9). It is unclear how many of α -coronaviruses utilize aminopeptidase N (APN) as their receptor. Where a SARS-CoV (β -coronaviruses) and HCoV-NL63 (α -coronaviruses) use ACE2 as their receptor, MHV enters through CEACAM1 receptor and MERS-CoV binds to dipeptidyl-peptidase 4 (DPP4) to gain entry into human cells (9) (Refer figure 3).

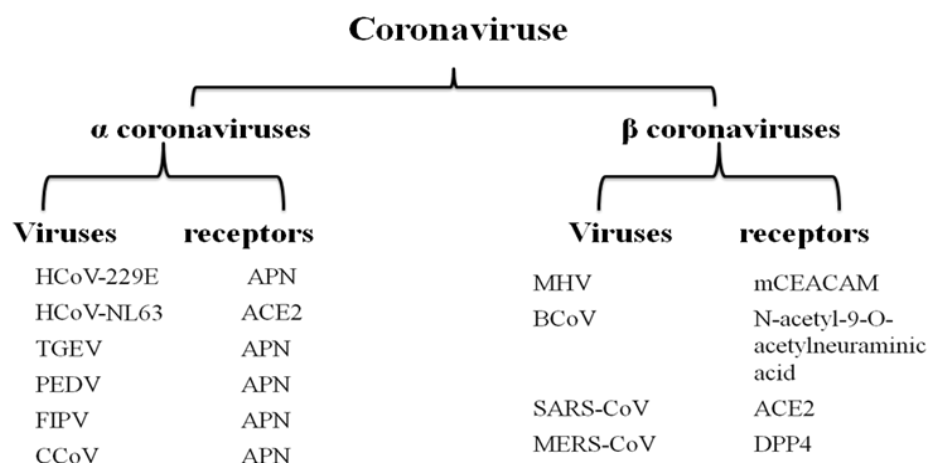


Figure 3. Flow chart showing α and β coronavirus and their associated receptors; APNaminopeptidaseN, **ACE2**angiotensin-converting enzyme 2,**mCEACAM** murine carcinoembryonic antigen-related adhesionmolecule 1,**DPP4**dipeptidyl peptidase 4,**HCoV**human coronavirus,**TGEV**transmissible gastroenteritis virus ,**PED V**porcine epidemic diarrhea virus, **FIPV** feline infectious peritonitis virus, **CCoV** canine coronavirus, **MHV** murine hepatitis virus, **BCoV** bovine coronavirus, **SARS-CoV** severe acute respiratory syndrome coronavirus, **MERS-CoV** Middle East respiratory syndrome coronavirus (9).

3.2 Viral genome replication and assembly

Fusion of viral envelope with host cell membrane results in the release of the viral genome into the cytoplasm. The viral genome uses replicase-transcriptase protein RNA dependent RNA polymerase (RdRp) to replication and transcription of RNA from a RNA strand (1,37). Viral RNA genome has 5' methylated cap and a3' polyadenylated tail bind to the host cell's ribosome for translation. Initially the viral genome also translates with host ribosome genome at the overlapping open reading frame and form long polyprotein. Mature polyproteins have own proteases to cleave the polyprotein into multiple nonstructural proteins (9). The replicase of viral genome encodes two large polyproteins (pp1a and pp1b), which encode non-structural protein (17) and form replication-transcription complex (RTC) in double-membrane vesicle (38). Continuously replications of RTC produce a nested set of sub genomic RNAs (39). Further, these RNAs encode accessory proteins and structural proteins. Once sufficient amount of structural proteins and viral RNA are formed, viral RNA then assembles with the viral structural proteins into virion. Now, viral assembly and budding occur in smooth walled vesicles in the endoplasmic reticulum, Golgi intermediate compartment (ERGIC) (40), and virion-containing vesicles fuse with the plasma membrane to release the virus through exocytosis, as shown in figure 4.

Protein-protein instruction via M protein plays an important role in virus assembly, but this is not enough for virus-like particles (VLPs) formation. M protein formed VLPs along with E protein. Also, these two proteins function together to produce coronavirus envelopes (41). Some literatures suggested that E protein prevents the aggregation of M protein (42,43), while others reported that E protein have role in promoting viral release by altering the host secretory pathway (44). Non-structural proteins (N) enhance the VLP formation and genomes encapsidation. S protein is not required for assembly but to traffic to the golgi intermediate compartment and interact with the M protein (41,42). It is still not clear either virus uses the traditional pathway for transport of large cargo from the golgi or has a separate, unique pathway for its own exit.

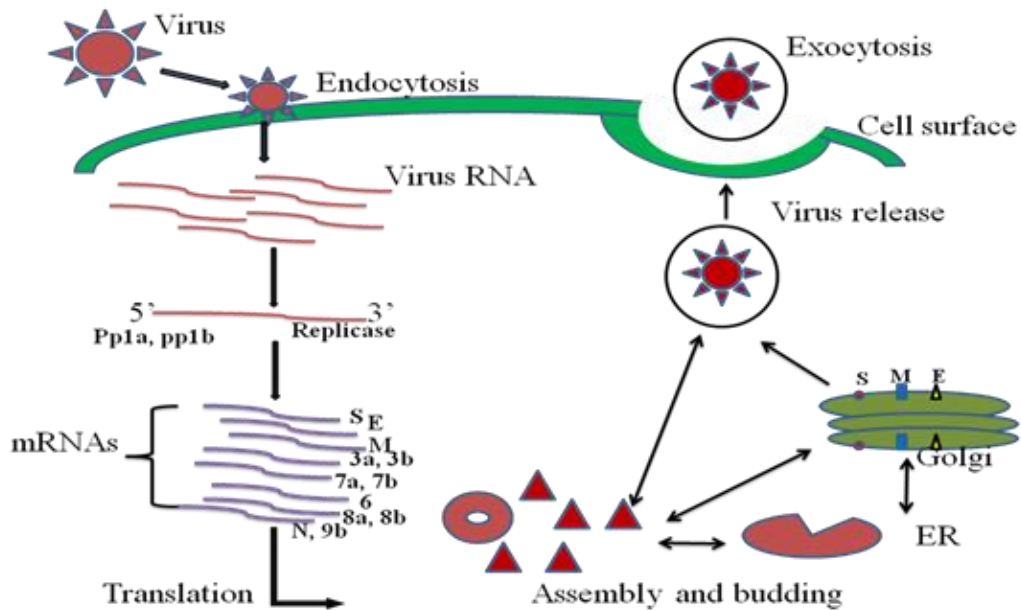


Figure 4. Life cycle of a COVID-19

4 Discussion

Novel coronavirus, COVID-19, was identified in January 2020 as the cause of an outbreak of viral pneumonia in Wuhan, China and spread worldwide (45,46). The Centers for Disease Control and Prevention (CDC) and National Institute of Allergy and Infectious Diseases (NIAID) developed a test to diagnose COVID-19 in respiratory and serum samples. Earlier NIAID built on research on severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS), which were also caused by coronaviruses. In above section, Introduction already described the information about COVID-19 (7,12). ICTV-CSG- International Committee on Taxonomy of Viruses- Coronavirus Study Group

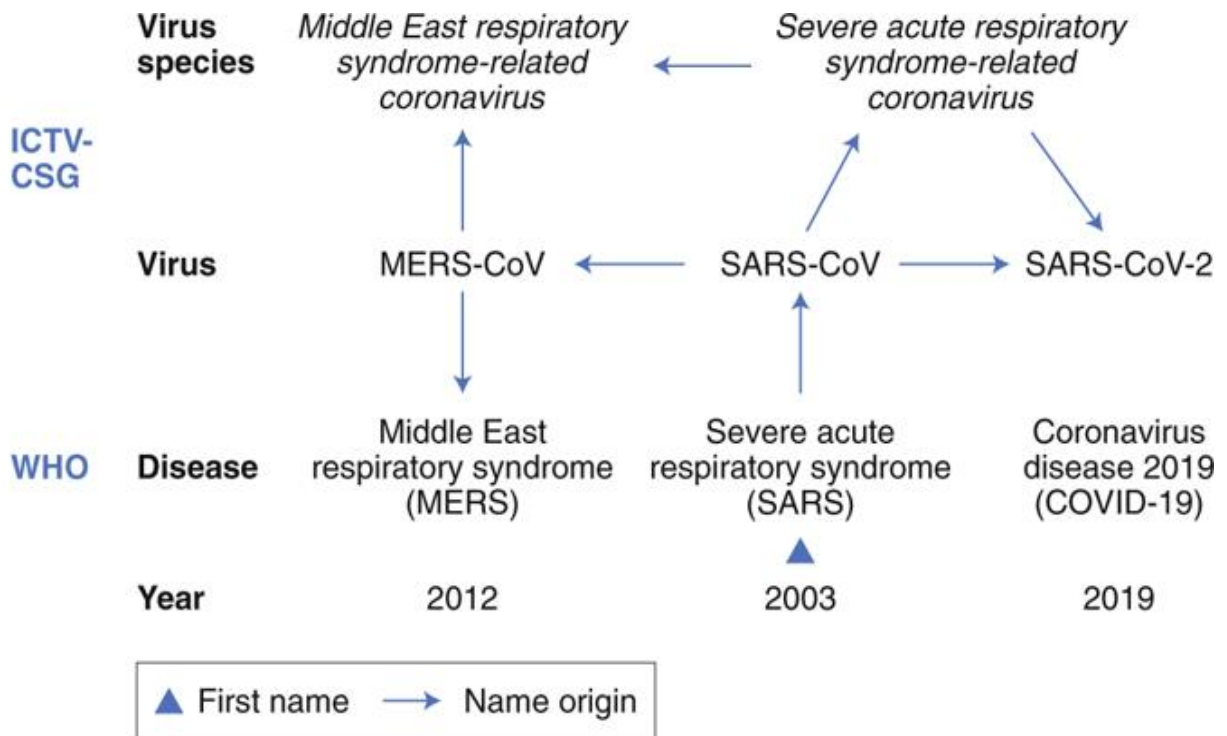


Figure 5. Flow chart showing systematic link between SARS-CoV, MERS-CoV, and COVID-19 (46).

Middle East respiratory syndrome (MERS-Cov), caused by the β -coronavirus, is a common-cold coronavirus with single-stranded RNA genomic structure (47). MERS-Cov was first reported in Saudi Arabia in September 2012 and had since spreaded to 27 countries (centers for disease control and prevention). MERS-CoV was believed to be originally from bats (48). As per WHO reported, people infected with MERS-CoV develop severe respiratory illness, fever, cough and shortness in breath (3,19). The humans were typically infected from camels, via direct or indirect contact. Spread between humans, typically required a close contact with an infected person. In January 2020, WHO confirmed 2,519 MERS cases and 866 deaths, in which 80% have occurred in Saudi Arabia and only two have tested positive for MERS-CoV in United States (48).

Severe acute respiratory syndrome coronavirus (SARS-CoV or SARS-CoV-1) causes severe acute respiratory syndrome (SARS) (49,50). SARS-CoV, belongs to β -coronavirus family, is a single-stranded RNA, positive-sense, enveloped virus and mostly infects the epithelial cells within the lungs. Virus uses host ACE2 receptor to enter the host cell (Figure 4). It infects humans, bats, and palm civets (51,52). SARS-CoV was first observed in Asia in February 2003 and subsequently was tracked back to November 2002 and quickly spread to 26 countries. It effected more than 8,000 people and 774 died. In April 2003, the Centers for Disease Control and Prevention (CDC) and National Microbiology Laboratory (NML) identified the SARS-CoV genome (53,54).

Research based studies suggests that both SARS-CoV and MERS-CoV originated from bats, and having similar characteristics like COVID-19 (Figure 5). Scientists are trying to determine how COVID-19 spread from an animal reservoir to people. Coronavirus pandemic of COVID-19 showed many similarities with SARS outbreak in 2002. The COVID-19 also has been also identified the similar characteristics with SARS-CoV (48,53,54). Strains of SARS-CoV including human coronavirus 229E(HCoV-229E), human coronavirus NL63(HCoV-NL63), human coronavirus OC43 (HCoV-OC43), human coronavirus HKU1(HCoV-HKU1), Middle East respiratory syndrome-related coronavirus (MERS-CoV), and COVID-19 (55,56).

5 Diagnosis and Treatment

There is no specific antiviral therapeutics/vaccine made till date that specifically target human coronaviruses (COVID-19), so treatments are only supportive. Some studies suggested that in-vitro interferons (IFNs) are partially effective against coronaviruses (57) including IFN-1, used as potential treatment of other positive ssRNA viruses. In-vitro recombinant INFs α and INFs β inhibit the replication of both SARS-CoV and MERS-CoV in animal models. Combination of both INFs α and INFs β along with other antivirals such as ribavirin can be used to treat patients with SARS or MERS, however, the effectiveness of this combination in-vivo requires further evaluation (57,58).

Vaccine approved for IBV (Infectious bronchitis virus), TGEV (Transmissible gastroenteritis virus), porcine epidemic diarrhea virus (PEDV, a vaccine for veterinary pathogens) and Canine CoV can be used as to prevent coronavirus infections, but these vaccines are not always used because they are not very effective. In some studies, it has been reported that these vaccines are involved in the selection of novel pathogenic COVIDs via recombination of circulating strains (59,50). There are several potential vaccines which have been developed viz., recombinant attenuated viruses, live virus vectors, or individual viral proteins expressed from DNA plasmids, but they are still waiting for approval for diagnosis of SARS-COVID (61,62).

Some studies have shown during this pandemic chloroquine (an anti-malarial drug, used to prevent or treat malaria) might be effective against COVID-19 (63,64). Researchers and scientists think that

chloroquine might help COVID-19 patients by inhibiting the coronavirus from entering the cell. India, USA, Spain and some other countries are widely using this drug prophylactically and have found some success in recovery of COVID-19 positive patients. The Danish Medicine Agency has refused to authorize Chloroquine for COVID-19. European Medicine Agency has announced that chloroquine should only be used for clinical trials (65). At present there are not enough scientific studies to draw any conclusion on the effect of Chloroquine drug on COVID-19.

Now a multiplex real-time RT-PCR assay has become the new choice for diagnosis of human COVID. Also, it is able to detect all four respiratory human COVIDs (66,67). RT-PCR is useful to detect the conserved RNA sequences of virus, but studies suggest that it needs more attention. Serologic assays are important in cases where RNA is difficult to isolate or is no longer stable, and for epidemiological studies. The best way to control or protect from this virus is via strong public health surveillance system coupled with rapid diagnostic testing and quarantine when necessary. Take healthy food to boost immune system. For international outbreaks, cooperation of governmental entities, public health authorities, and health care providers is critical. The WHO and other organizations have issued the following general recommendations (68).

- Avoid close contact with infected patients.
- Wash hands frequently, especially after contact with infected person.
- Avoid unprotected contact with farm or wild animals.
- People with symptoms of acute airway infection should keep their distance, cover coughs or sneezes with disposable tissues or clothes and wash their hands.
- Strengthen, in particular, in emergency medicine departments, the application of strict hygiene measures for the prevention and control of infections.
- Individuals that are immune compromised should avoid public gatherings.

6 Conclusion

Several research investigations have identified that COVID-19 belongs to β -coronavirus family with positive sense RNA genome and has a highly identical genomic structure to bat coronavirus. The novel COVID-19 uses ACE2 (Angiotensin-converting enzyme 2) receptor as a binding receptor for their entry to human host cell, and mainly spreads through the respiratory tract. Several protein including S, M and E played an important role in virus packaging and assembling. Through a planned endocytosis and exocytosis pathway virus easily replicated their life cycle inside the living cell and transmitted to other cell. Till date, there is no specific antiviral therapeutics/vaccine made that are specifically target human coronaviruses (COVID-19), so treatments are only supportive. Studies suggest that interferon's (IFNs) are partially effective against coronaviruses. Vaccines including IBV, TGEV, PEDV are effective in some cases, but are not always used. Some studies have shown during this pandemic chloroquine might be effective against COVID-19. Molecular technique RT-PCR might be a useful tool to help to diagnose the COVID-19, but still needs more improvement. The outbreak of COVID-19 swept across China rapidly and has spread to 213 countries and killed more the 3,17,174 people with 6.57% fatality rate. Research studies suggest that both SARS-CoV and MERS-CoV were originated from bats, and have similar characteristics like COVID-19. Scientists have made progress in the characterization of the novel coronavirus and are working extensively on the therapies and vaccines against COVID-19. Till that, the best way to control or protect from this virus is via strong public health surveillance system coupled with rapid diagnostic testing and quarantine when necessary.

7 Competing Interests

The authors declared that no conflict of interest exists.

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