AIJR Preprints
Section: Coronavirus
Article Id: 79, Version: 1, 2020
URL:https://preprints.aijr.org/index.php/ap/preprint/view/79

{Click on above link to see the latest available version of this article}

NOT PEER-REVIEWED

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

Lalit Mohan Jeena*, Nidhi Singh, Anjali Tempe

Department of Gynaecology and obstructive, IVF Centre, Maulana Azad Medical College, New Delhi-110002

*Address for correspondence author: Dr Lalit Mohan Jeena, Department of Gynaecology and Obstructive, IVF Centre, Maulana Azad Medical College, New Delhi-110002. E-mail address: **********; 7805869142

Version 1: Received: 18 May 2020 / Approved: 20 May 2020 / Online: 21 May 2020

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 21thcentury. It has spread throughout China and is received as a pandemic worldwide. Till date (18thMay, 2020), a total of 4,827,272patients are infected and more than 3,17,174confirmed 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 constitutant of the subfamily Orthocoronavirinae (subgenus*sarbecovirus)*, 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 229Eandhuman coronavirus OC43(6). Other human coronaviruses have been involved in serious respiratory tract infections including SARS-CoV in 2003, HCoV NL63in 2004, HKU1 in 2005, MERS-CoV in 2012, andCOVID-19in 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 ofβ-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 18thMay 2020, a total 4,827,272patientshave been infected, while roughly 1,68,532 have recovered and 3,17,174have 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 mostinfectedcountrieswithCOVID-19.

Table1: *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	1,76788	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) andnucleocapsid (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 a5' methylated cap and a3' polyadenylated tail. Coronaviruses have a diameter of about 120nm (9).

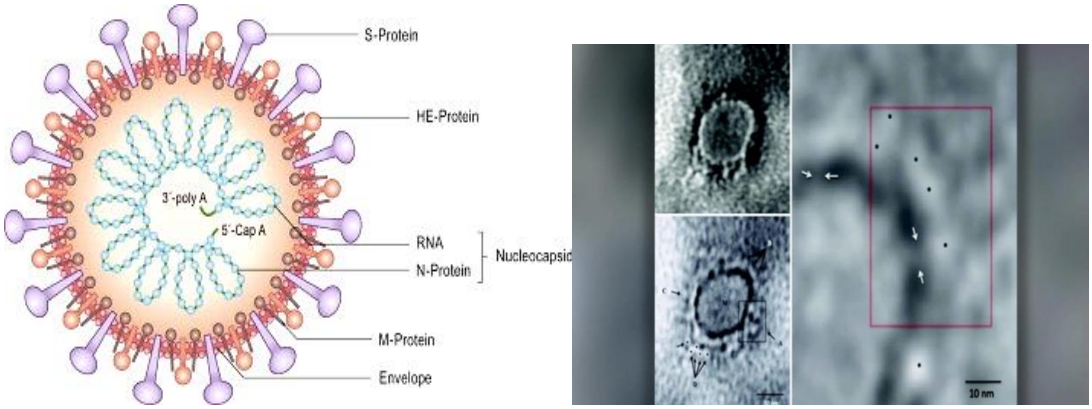

Figure 1. (a). Structure of coronavirus (69) **(b).** Transmission electron microscopy imaging of COVID-19 (71)

Figure1(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.

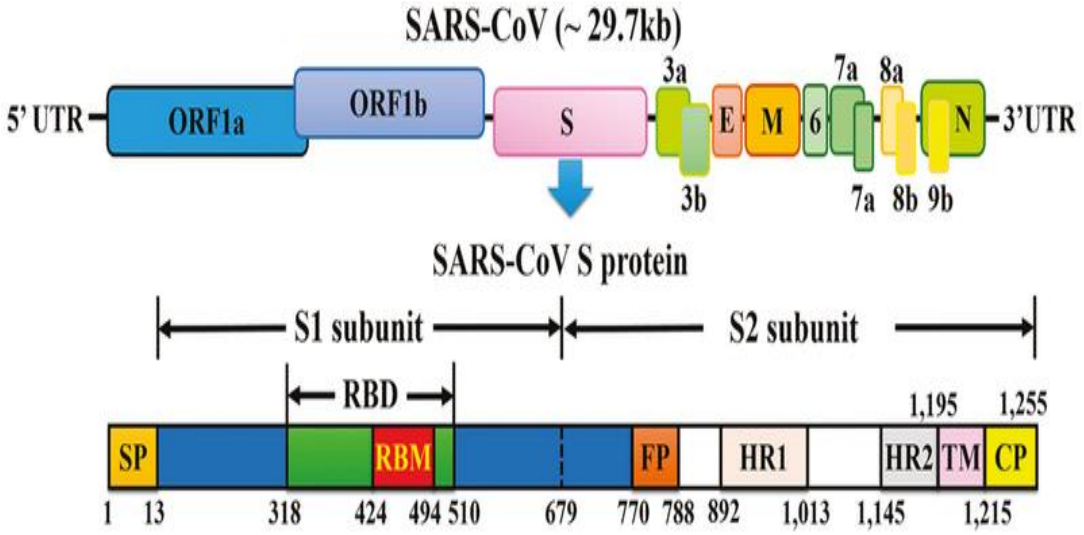

Figure 2. Genomic organization of COVID-19 (3).

The genome of coronavirus consists5'-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).

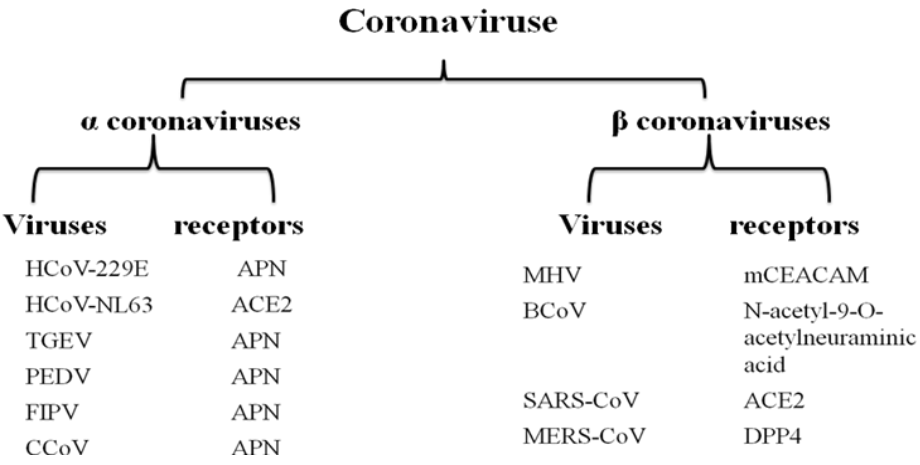

Figure 3. Flow chart showing α and β coronaviruse and their associated receptors; **APN**aminopeptidaseN, **ACE2**angiotensin-converting enzyme 2,**mCEACAM** murine carcinoembryonic antigen-related adhesionmolecule 1,**DPP4**dipeptidyl peptidase 4,**HCoV**human coronavirus,**TGEV**transmissible gastroenteritis 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 secretary 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.

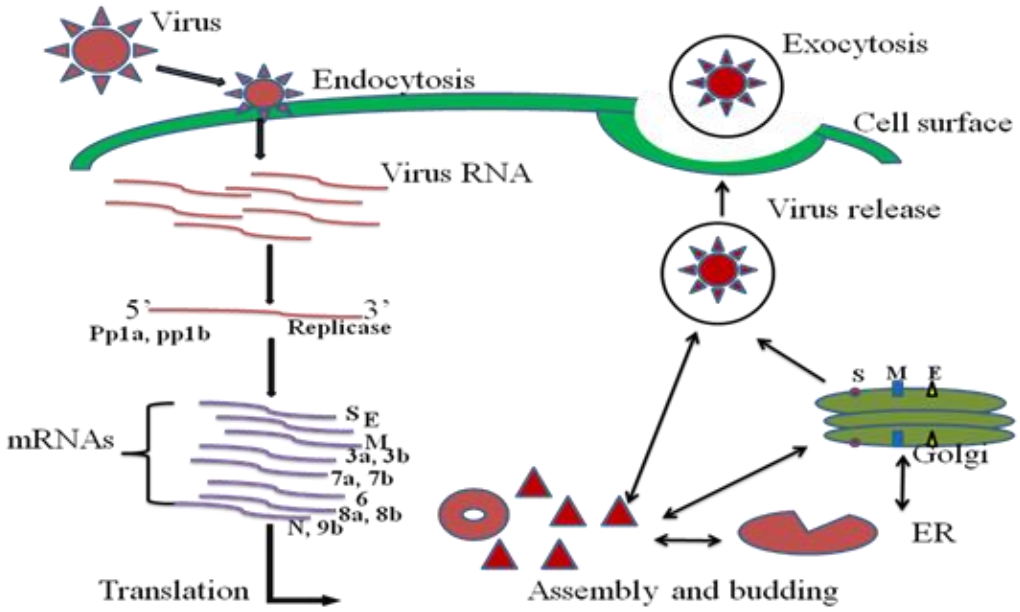

Figure 4. Life cycle of a COVID-19

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 (CDCP) and National Institute of Allergy and Infectious Diseases (NIAID) developed a test to diagnose COVID-19 in respiratory and serum samples. Earlier NIAID build 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 descried the information about COVID-19 (7,12). ICTV-CSG- International Committee on Taxonomy of Viruses- Coronavirus Study Group

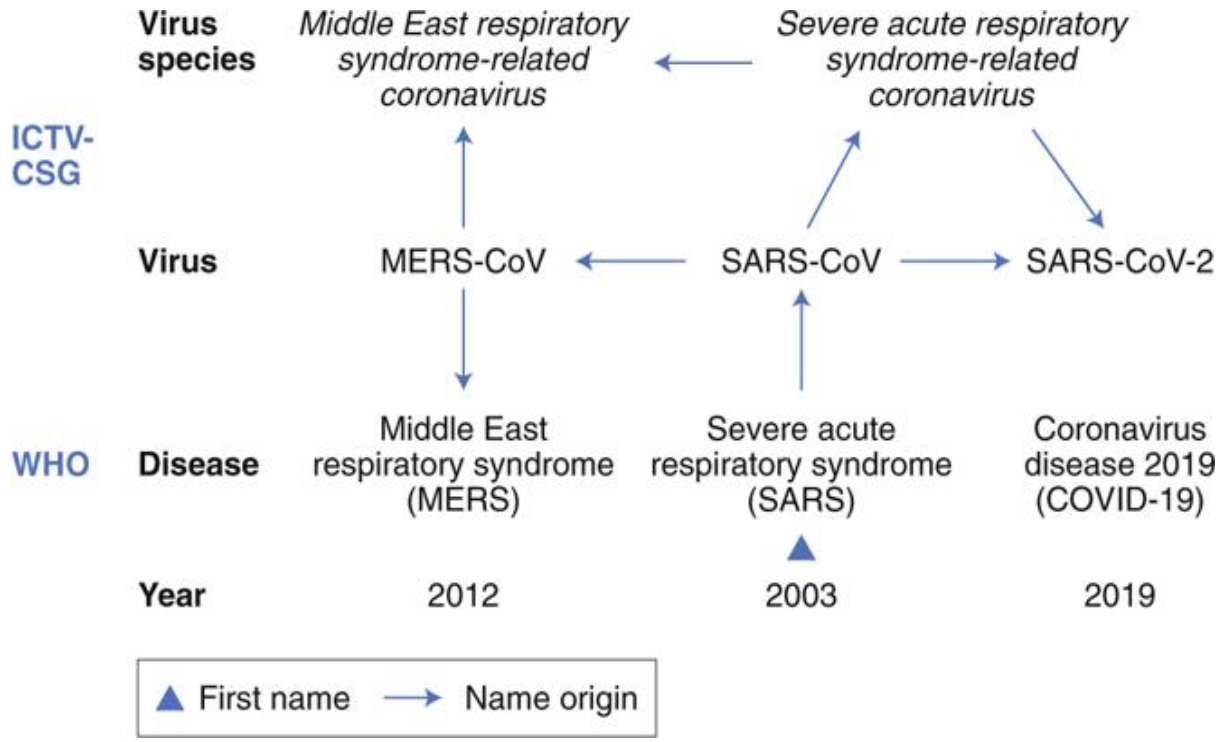

Figure 5. Flow chart showing systematic link between SARS-CoV, MERS-CoV, and COVID-19 (46).

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 toβ-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 novelCOVID-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.

How to Cite:

Lalit Mohan Jeena *et al.* Molecular Mechanism of Coronaviruses (COVID-19) and Diagnostic Approaches: A Systematic Review, *AIJR Preprints*,79, version 1, 2020. https://preprints.aijr.org/index.php/ap/preprint/view/79

References

(1). Sexton NR, Smith EC, Blanc H, Vignuzzi M, Peersen OB, Denison MR. Homology-Based Identification of a Mutation in the Coronavirus RNA-Dependent RNA Polymerase That Confers Resistance to Multiple Mutagens.J Virol. 2016;90(16):7415–7428. Published 2016 Jul 27. doi:10.1128/JVI.00080-16.

(2). de Groot RJ, Baker SC, Baric RS, et al. Middle East respiratory syndrome coronavirus (MERS-CoV): announcement of the Coronavirus Study Group.J Virol. 2013;87(14):7790–7792. doi:10.1128/JVI.01244-13.

(3). Zhiqi S et al. From SARS to MERS, Thrusting Coronaviruses into the Spotlight. Viruses.2019 Jan;11(1): 59.doi: 10.3390/v11010059.

(4). Kampf G, Todt D, Pfaender S, Steinmann E. Persistence of coronaviruses on inanimate surfaces and its inactivation with biocidal agents. J Hosp Infect 2020;195-6701.

(5). McIntosh K, Arber W, Haas R, Henle W, Hofschneider PH, Jerne NK, Koldovský P, Koprowski H, Maaløe O, Rott R. "Coronaviruses: A Comparative Review". Current Topics in Microbiology and Immunology / Ergebnisse der Mikrobiologie und Immunitätsforschung. Berlin, Heidelberg: Springer 1974;87-119.

(6). Geller C, Varbanov M, Duval RE. Human coronaviruses: insights into environmental resistance and its influence on the development of new antiseptic strategies. Viruses 2012;4(11):3044-68.

(7). Guo Y, Cao Q, Hong Z. et al. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak-an update on the status. Military Med Res 7,11:2020. https://doi.org/10.1186/s40779-020-00240-0.

(8). Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterization and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet 2020;22-28 February; 395(10224): 565–574.

(9). Fehr AR, Perlman S. Maier HJ, Bickerton E, Britton P (eds.). Coronaviruses: an overview of their replication and pathogenesis. Methods in Molecular Biology. Springer Science & Business Media New York 2015;1282:1-23.

(10). De GRJ, Baker SC, Baric R, et al. Family Coronaviridae, In King AM, Lefkowitz E, Adams MJ, Carstens EB, International Committee on Taxonomy of Viruses, International Union of Microbiological Societies 2011. Virology Division (eds.). Ninth Report of the International Committee on Taxonomy of Viruses. Oxford: Elsevier. pp. 806-28.

(11). Covid-19 coronavirus pandemic. 18th May 2020. https://www.worldometers.info/coronavirus/cuntries.

(12). Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A novel coronavirus from patients with pneumonia in China. N Engl J Med 2020;382(8):727–33.

(13). Lai MM, Cavanagh D. The molecular biology of coronaviruses. Advances in Virus Research 1997;48:1-100.

(14). International Committee on Taxonomy of Viruses. ICTV Master Species List 2009-v10;2010-08-24.

(15). Saberi A, Gulyaeva AA, Brubacher JL, Newmark PA, Gorbalenya AE. A planarian nidovirus expands the limits of RNA genome size. PLoSPathog 2018; 14(11): e1007314

(16). McBride R, Van ZM, Fielding BC. The coronavirus nucleocapsid is a multifunctional protein 2014;Aug7:6(8):2991-3018.

(17). Millet JK, Whittaker GR.Host cell proteases: Critical determinants of coronavirus tropism and pathogenesis. Virus Res. 2015;16: 202:120-34.

(18). Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003;426(6965):450–454. doi:10.1038/nature02145.

(19). Mou H, Raj VS, van Kuppeveld FJ, Rottier PJ, Haagmans BL, Bosch BJ. The receptor binding domain of the new Middle East respiratory syndrome coronavirus maps to a 231-residue region in the spike protein that efficiently elicits neutralizing antibodies. J Virol. 2013;87(16):9379-83.

(20). Xiao X, Chakraborti S, Dimitrov AS, Gramatikoff K, Dimitrov DS. The SARS-CoV S glycoprotein: Expression and functional characterization. Biochem. Biophys. Res. Commun. 2003; 312:1159-1164.

(21). Bosch BJ, Bartelink W, Rottier PJ, Cathepsin L. functionally cleaves the severe acute respiratory syndrome coronavirus class I fusion protein upstream of rather than adjacent to the fusion peptide. J Virol Sep 2008;82 (17):8887-8890.

(22). Armstrong J, Niemann H, Smeekens S, Rottier P, Warren G. Sequence and topology of a model intracellular membrane protein, E1 glycoprotein, from a coronavirus. Nature. 1984;308(5961):751–752. doi:10.1038/308751a0.

(23). Nal B, Chan C, KienF,etal.Differential maturation and subcellular localization of severe acute respiratory syndrome coronavirus surface proteins S, M and E. J Gen Virol 2005;86(5):1423-1434.

(24). Godet M, L'Haridon R, Vautherot JF, Laude H. TGEV corona virus ORF4 encodes a membrane protein that is incorporated into virions. Virology 1992;188(2):666-75.

(25). DeDiego ML, Alvarez E, Almazán F, Rejas MT, Lamirande E, Roberts A, Shieh WJ, Zaki SR, Subbarao K, Enjuanes L. J Virol. A severe acute respiratory syndrome coronavirus that lacks the E gene is attenuated in-vitro and in-vivo. J Virolo2007;81(4):1701-13.

(26). Chang CK, Sue SC, Yu TH, Hsieh CM, Tsai CK, Chiang YC, Lee SJ, Hsiao HH, Wu WJ, Chang WL, Lin CH, Huang TH. Modular organization of SARS coronavirus nucleocapsid protein. J Biomed Sci 2006;13(1):59-72.

(27). Hurst KR, Koetzner CA, Masters PS. Identification of in-vivo interacting domains of the murine coronavirus nucleocapsid protein. J Virol 2009;83(14):7221-34.

(28). Hurst KR, Koetzner CA, Masters P. Characterization of a critical interaction between the coronavirus nucleocapsid protein and nonstructural protein 3 of the viral replicase-transcriptase complex. J Virol 2013;87(16):9159-72.

(29). Sturman LS, Holmes KV, Behnke Isolation of coronavirus envelope glycoproteins and J interaction with the viral nucleocapsid. J Virol 1980;33(1):449-62.

(30). Angeletti S, Benvenuto D, Bianchi M, Giovanetti M, Pascarella S, Ciccozzi M. COVID-2019: the role of the nsp2 and nsp3 in its pathogenesis. J Med Virol 2020;92: 584-588.

(31). Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, Graham BS, McLellan JS. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science 2020;13;367(6483):1260-1263.

(32). Tang X, Wu C, Li X, et al. On the origin and continuing evolution of SARS-CoV-2. Natl Sci Rev. 2020;nwaa036. Published 2020 Mar 3. doi:10.1093/nsr/nwaa036

(33). Saberi A, Gulyaeva AA, Brubacher JL, Newmark PA, Gorbalenya AE. A planarian nidovirus expands the limits of RNA genome size. PLoSPathog 2018; 14(11): e1007314.

(34). Donoghue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, Donovan M, Woolf B, Robison K, Jeyaseelan R, Breitbart RE, and Acton S (1 Sep 2000). A Novel Angiotensin-Converting Enzyme–Related Carboxypeptidase (ACE2) Converts Angiotensin I to Angiotensin 1-9. Circulation Research 2000;87:e1–e9.

(35). Letko M, Marzi A, Munster V. Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses. Nat Microbiol 2020; 5;562-569.

(36). Song W, Gui M, Wang X, Xiang Y. Cryo-EM structure of the SARS coronavirus spike glycoprotein in complex with its host cell receptor ACE2. PLoSPathog 2018; 14(8):e1007236.

(37). Simmons G, Zmora P, Gierer S, Heurich A, Pöhlmann S. Proteolytic activation of the SARS-coronavirus spike protein: cutting enzymes at the cutting edge of antiviral research. Antiviral Research 2013;100(3): 605-14.

(38). Sawicki SG, Sawicki DL. Coronavirus transcription: a perspective. Curr Top Microbiol Immunol 2005; 287:31-55.

(39). Hussain S, Pan J, Chen Y, *et al*. Identification of novel subgenomic RNAs and noncanonical transcription initiation signals of severe acute respiratory syndrome coronavirus. J Virol May 2005;79(9):5288-95.

(40). Masters, P.S. The molecular biology of coronaviruses. Adv. Virus Res 2006; 66:193-292.

(41). Bos EC, Luytjes W, van der Meulen HV, Koerten HK, Spaan WJ. The production of recombinant infectious DI-particles of a murine coronavirus in the absence of helper virus.Virology 1996;1:218(1):52-60.

(42). Siu YL, Teoh KT, Lo J, et al. The M, E, and N structural proteins of the severe acute respiratory syndrome coronavirus are required for efficient assembly, trafficking, and release of virus-like particles. J Virol 2008 Nov;82(22):11318-11330.

(43). Boscarino JA, Logan HL, Lacny JJ, Gallagher TM.Envelope protein palmitoylations are crucial for murine coronavirus assembly.J Virol 2008;82(6):2989-99.

(44). Ye Y & Hogue BG. Role of the coronavirus E viroporin protein transmembrane domain in virus assembly. J Virol 2007Apr ;81(7):3597-3607.

(45). Drosten C, Gunther S, Preiser W, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N Engl J Med 2003 May;348(20):1967-1976.

(46). Gorbalenya AE, Baker SC, Baric RS *et al*. The species severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol 2020; 5:536-544.

(47). Saey TH. Story one: Scientists race to understand deadly new virus: SARS-like infection causes severe illness, but may not spread quickly among people. Science News 2013;183(6):5-6.

(48). Middle East respiratory syndrome coronavirus (MERS-CoV). www.who.int. Retrieved 15 April 2020. https://www.who.int/csr/don/08-april-2020-mers-saudi-arabia/en/

(49). Neeltje VD, Trenton B, Dylan H, et al. Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1. N Engl J Med 2020; 382:1564-1567.

(50). Leibowitz JL. Coronaviruses: Molecular and Cellular Biology. Emerg Infect Dis. 2008;14(4):693–694. doi:10.3201/eid1404.080016

(51). Wong ACP, Li X, Lau Susanna KP, Woo Patrick CY. Global Epidemiology of Bat Coronaviruses. Viruses2019;11(2):174.

(52). Li F. Receptor recognition and cross-species infections of SARS coronavirus. Antiviral research. 2013 Oct;100(1):246-254.

(53). Remembering SARS: A Deadly Puzzle and the Efforts to Solve It. Centers for Disease Control and Prevention. Archived from the original on 2013. Retrieved 2013-08-03. https://www.cdc.gov/about/history/sars/feature.htm

(54). Coronavirus never before seen in humans is the cause of SARS. United Nations World Health Organization. Archived from the original on 2004-08-12. Retrieved 2006-07-05. https://news.un.org/en/story/2003/04/64992-who-announces-discovery-virus-causes-sars.

(55). Woo PC, Lau SK, Chu CM, et al. Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. J Virol. 2005 Jan;79(2):884-895.

(56). Vander HL, Pyrc K, Jebbink MF, et al. Identification of a new human coronavirus. Nat Med. 2004 Apr;10(4):368-373.

(57). Cinatl J, Morgenstern B, Bauer G, Chandra P, Rabenau H, Doerr HW. Treatment of SARS with human interferons. Lancet. 2003 Jul;362(9380):293-4.

(58). Stockman LJ, Bellamy R, Garner P. SARS: systematic review of treatment effects.PLoS Med.2006;3:e343.

(59). Laude H, Van Reeth K, Pensaert M. Porcine respiratory coronavirus: molecular features and virus-host interactions.Vet Res1993;24(2):125-150.

(60). Saif LJ. Animal coronavirus vaccines: lessons for SARS.Dev Biol (Basel)2004;119:129-140.

(61). He H Y, Tang X, Qin W, Xu Y, Wang X, Liu X, Liu S, Xiong J, Li MZ, Duan M**.** Construction of a eukaryotic expressionplasmidencoding partial S gene fragments of the SARS-CoV and its potential utility as a DNA vaccine.DNA Cell Biol.2005 Aug;24(8)**:**516-520.

(62). He Y, Zhou Y, Liu S, Kou Z, Li W, Farzan M, Jiang S. Receptor-binding domain of SARS-CoV spike protein induces highly potent neutralizing antibodies: implication for developing subunitvaccine.Biochem. Biophys. Res. Commun.2004 Nov;324(2)**:**773-781.

(63). Savarino A, Boelaert JR, Cassone A, Majori G, Cauda R. Effects of chloroquine on viral infections: an old drug against today's diseases?.Lancet Infect Dis. 2003;3(11):722–727. doi:10.1016/s1473-3099(03)00806-5

(64). Al-Bari MAA. Targeting endosomal acidification by chloroquine analogs as apromoting strategy for the treatment of emerging viral disease. Pharamcol Res Prespect. 2017;5(1):e00293.

(65). EMA clarifies use of chloroquine and hydroxychloroquine for COVID-19; 2April 2020. https://laegemiddelstyrelsen.dk/en/news/2020/ema-clarifies-use-of-chloroquine-and-hydroxychloroquine-for-covid-19.

(66). Emery SL, Erdman DD, Bowen MD, et al. Real-time reverse transcription-polymerase chain reaction assay for SARS associated coronavirus.Emerg Infect Dis2004;10(2):311-316.

(67). Gaunt ER, Hardie A, Claas EC, *et al*. Epidemiology and clinical presentations of the four human coronaviruses 229E, HKU1, NL63, and OC43 detected over 3 years using a novel multiplex real-time PCR method.J Clin Microbiol2010;48(8):2940-2947.

(68). Updated WHO recommendations for international traffic in relation to COVID-19 outbreak. 29 February 2020. https://www.who.int/news-room/articles-detail/updated-who-advice-for-international-traffic-in-relation-to-the-outbreak-of-the-novel-coronavirus-2019-ncov.

(69). J.S.M. Peiris, in Medical Microbiology (Eighteenth Edition), 2012. https://www.sciencedirect.com/topics/neuroscience/coronavirus.

(70). Johns Hopkins, University & Medicine. New cases of COVID-19 in world countries. 18th May 2020. https://coronavirus.jhu.edu/data/new-cases.

(71). Transmission electron microscopy imaging of SARS-CoV-2. Indian Journal of Medicine Research. March 2020, pp 241-243. DOI: 10.4103/ijmr.IJMR_577_20