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Coronaviruses: A Review of the Genetics and Proteins Associated with the Life Cycle of SARS-CoV-2

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Abstract

The history of coronaviruses dates back to the 1960s. There have been several coronaviruses induced epidemics such as severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) in the recent past. More recently, another coronavirus induced disease, namely COVID-19 emerged as an epidemic and rapidly developed into a pandemic due to the high transmissibility of SARS-CoV-2. It emerged as an epidemic novel COVID-19, in late 2019, instigated by SARS-CoV2. This review analyses the different aspects of SARS-CoV-2 including its genomic structure, protein composition, transmission mode, and life cycle. SARS-CoV-2 is an RNA virus, which codes four structural proteins along with various accessory proteins. A unique property of COVID-19 is that it incorporates a polybasic cleavage site, which increases its pathogenicity. The genomic variation of COVID-19/SARS-CoV-2 is assumed to be the reason behind its high transmissibility. It was identified that this genomic variation hinders the development of treatment against this disease. This review aims to facilitate the prevention of this infectious disease as well as suggest possible treatment regimens.

Keywords: COVID-19, genomic variation, pandemic, proteins, SARS-CoV-2

Introduction

Coronaviruses are a part of Nidovirales order and pertain to the Coronaviridae family. The term corona comes from the Latin word *corona*

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which means "crown" or "halo." World Health Organization (WHO) stated that Coronaviruses are a category of viruses that cause the common cold and other serious illnesses such as severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS) [1].

By the end of 2019, a new coronavirus was reported in Wuhan, China. It spread rapidly within the first 50 days of the epidemic and infected over seventy thousand individuals, out of which more than 1800 individuals died [2]. The new coronavirus SARS-COV2 was discovered by Chinese scientists on 7th January 2020. It was assumed that the virus was transmitted to people via the seafood market, where it spread from one person to another. Additionally, it was identified that the exposure of healthy individuals to patients of COVID-19, who are frequently coughing, sneezing, and dropping aerosols, causes the virus to spread rapidly. Inhalation through the nose or mouth allows aerosols to enter the human body [3].

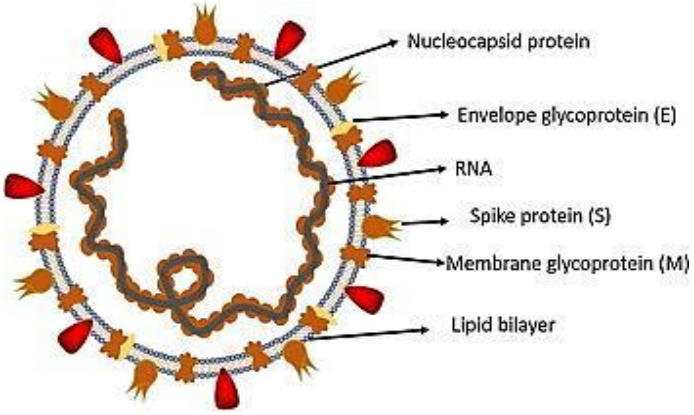
In order to stop the virus from spreading, it is crucial to understand the viral genome and protein structure of SARS-CoV2. With the help of high throughput sequencing technologies, it was deciphered that the RNA sequence of the coronavirus (SARS-CoV2) genome is 30,000 bases long and has a specific polybasic cleavage site. The SARS-CoV2 virion structure is around 50–200 nanometers in diameter and constitutes/comprises four structural proteins, namely S (spike), E (envelope), M (membrane), and N (nucleocapsid). The N protein is involved in synthesisthe RNA genome, while the rest of the proteins are involved in the formation of a viral envelope [4].

The spike (S) protein binds with the cell surface receptors, which allows the virus to be attached with the host cell membrane. Thus, this protein facilitates the integration of the viral genome [5]. Coronaviruses also include/are also composed of an important glycoprotein called Hemagglutinin acetyl esterase (HE), which binds to sugar moieties in cell membranes (Figure 1) [6].

This review aimed to understand different aspects of coronavirus including its genomic organization, mode of transmission, proteins of

coronavirus, life cycle, and possible ways of transmission to help prevent its spread [7].

Figure 1. Covid-19 Virus Structure (Adopted from Science Direct)



Organization of the Genome

Coronaviruses are non-segmented positive-sense RNA viruses with a genomic size of 30 kb, they are composed of 29899 nucleotides and 9860 amino acids. Their genomic RNA has a 5′ cap and a 3′ poly (A) tail and therefore can be used as mRNA for replicase polyprotein translation [8]. The organized structure starts from a 5' UTR leader-replicase-S gene–E gene–M gene–N gene and ends with a 3′UTR-poly (A) tail [9]. UTR region at 5′ end of genome is required for the replication and transcription of RNA, which has multiple stem-loop structures. On the other hand, the UTR region at the 3′ end has RNA structures, which are critical for viral RNA replication and synthesis [10].

In January 2020, the novel virus SARS-CoV2 was sequenced and published for the very first time. ORF1a and ORF1b are open reading frames (ORFs) that translate 440-500kDa (pp1a) and 740-810kDa (pp1ab) polypeptides, respectively. They make up two-thirds of the genome and have a combined length of 21290 bp. The length of Spike (S), envelope (E), membrane (M), and nucleocapsid (N) are 3822bp, 228bp, 669bp, and 908bp long, respectively. Alternatively, in comparison to structural proteins, six accessory proteins have been identified to be shorter in length

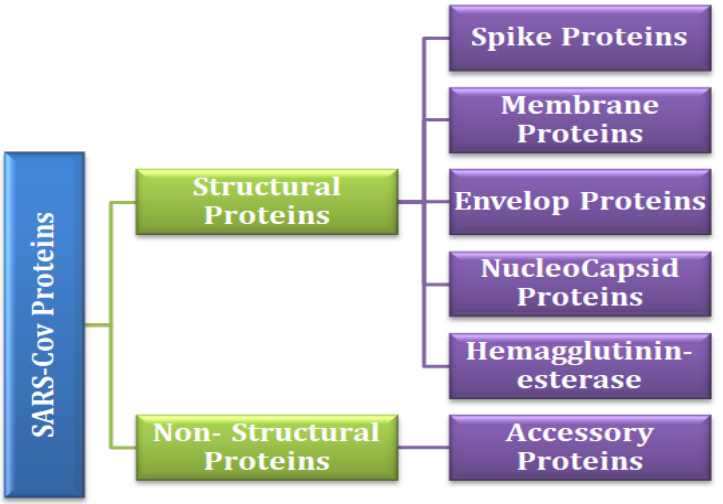
[11-12]. The overall genomic organization with ORF regions at polyproteins is depicted in Figure 2.

Figure 2. Genome Organization of SARS-CoV2

Categorization of Coronaviruses Proteins

The coronavirus protein composition is divided into structural and non-structural proteins. (Figure 3) [13].

Figure 3. SARS-CoV Proteins



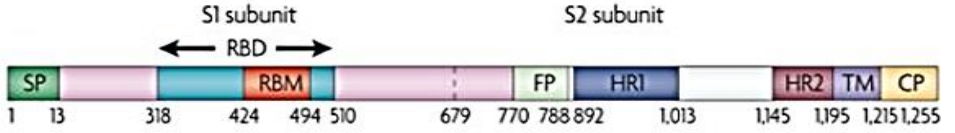
Structural Proteins

The structural genes encode spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins [14].

Spike Glycoprotein

Spike protein (S protein) is about 150 kDa in size and has a glycosylated N-terminal signal region that is essential for entry into ER (endoplasmic reticulum). Additionally, S protein encoded by viral homotrimers generates various spike shapes on the viral surface [15]. There are 1255 amino acids in S protein and 23 possible N-linked glycosylation sites. A class I fusion protein that can attach to a host receptor is known as trimeric spike glycoprotein. The host cell's furin-like protease divides the S protein into two polypeptides, namely S1 and S2 [16]. S1 forms the spike protein's receptor-binding domain (RBD), which allows the virus to adhere to the host cell; whereas, S2 creates the spike protein's stalk [17]. The genomic architecture of spike protein along with their sub-structures is summarised in Figure 4.

Figure 4. The SARS-CoV2 Spike Protein's Genetic Architecture (Adopted from Research Gate)



Membrane Protein

The most prominent structural protein observed in the virion was a 25–30 kDa transmembrane protein, consisting of 222 amino acids [18] These acids give the protein its virion shape. The protein is composed of a small glycosylated N-terminal ectodomain and a comparatively larger C-terminal endodomain [19]. Its membrane protein lacks a signal sequence and is found in the virion as a dimer with two distinct conformations, which allow the protein to create membrane curvature and nucleocapsid binding.

Envelope Protein

The virion contains a modest amount of the 8–12 kDa envelope protein. Envelope polypeptide is a transmembrane protein with an ectodomain at the N-terminus, an endodomain at the C-terminus, and an ion channel. It aids in the assembly and release of the virus, as well as performing a

variety of other tasks. The ion channel sub-unit is not necessary for the replication of SARS-CoV2 but is essential for disease transmission [20].

Nucleocapsid Protein

An N-terminal domain (NTD) and a C-terminal domain (CTD) make up a nucleocapsid protein. Both of these domains attach and combine with RNA. However, every domain attaches to RNA in a distinct/unique way [21]. The two distinct RNA substrates for nucleocapsid protein are transcription regulatory sequences (TRSs) and genomic packing signals. The packaging signal of the genome attaches to the CTD of RNA as well as to nsp3 [22] as a cellular membrane and a member of the replicase complex. These protein associations assist the viral genome in tying/to attach with the reverse transcription complexes (RTCs) and packing the enclosed genome into virion [23].

Hemagglutinin-esterase

Hemagglutinin-esterase (HE) acts like hemagglutinin and is found in several coronaviruses. Once this enzyme get acetylated, it mediates the reversible attachment of *O*-acetylated sialic acids on surface glycoproteins [24]. These actions are critical since they increase spike protein-mediated cell entrance and virus propagation through mucus [25].

Non-structural Proteins

Several genes synthesize different proteins needed for viral replication, transcription, and assembly. These non-structural proteins are synthesized by translation of two important open reading frames 1a and 1b (ORF1ab) [26]. These proteins play a significant role in viral RNA genome replication and transcription. Other than the structural and accessory proteins, some other genes that are present are known as “group-specific or accessory genes”. These proteins are essential for viral survival in the infected host's natural environment [27,28].

Several accessory genes are present near the 3′-end region of the genome. Additionally, other accessory or group-specific genes are widely scattered throughout the genome including two genes in-between S and E genes (ORFs 3a and 3b,) five genes in-between M and N genes (6, 7a, 7b, 8a, and 8b), and one gene inside the N gene (9b) of the genome [29,30]. In

Figure 5, all these accessory proteins and their expected functions are enlisted.

Table 1. Summarization of SARS-CoV Accessory Proteins (Adopted from Science Direct)

Accessory Proteins	Incorporation into Viruses	Functions
3a	Yes	NF↑, JNK ↑, IL-8 ↑, RANTES ↑ Ion Channel activity, apoptosis induction and cell cycle arrest.
3b	Unknown	Type 1 INF production and signalling inhibition, apoptosis induction and cell cycle arrest
ORF6	Yes	Type 1INF production and signalling inhibition
7a	Yes	NF↑, JNK ↑, IL-8 ↑, p38 MAP knase, host translation inhibition, apoptosis induction and cell cycle arrest
7b	Yes	No known function
8a	Unknown	No known function
8b	Unknown	No known function
9b	Unknown	No known function

Most of the genes sequences in human and animal SARS-CoV are conserved. Nevertheless, as anticipated, these clusters of genes have no resemblance to accessory genes found in other coronavirus groups [31]. An overall genome organization of coronaviruses as well as of SARS-CoV2 is shown in Figures 6 and 7 respectively.

Figure 5. Beta Coronaviruses Genome Organization (Adopted from Science Direct)

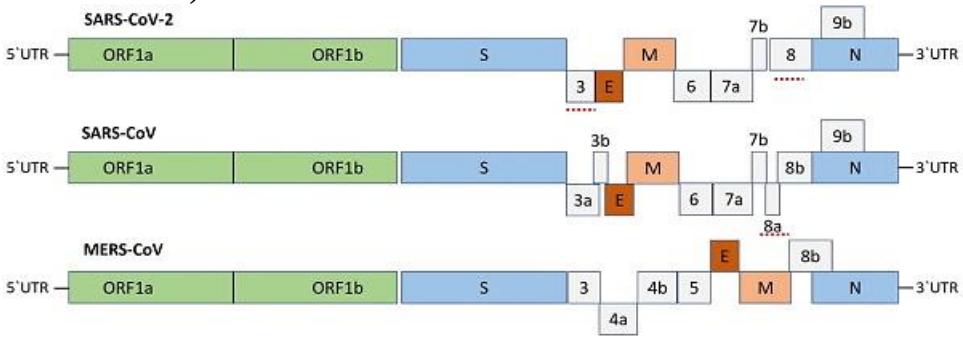
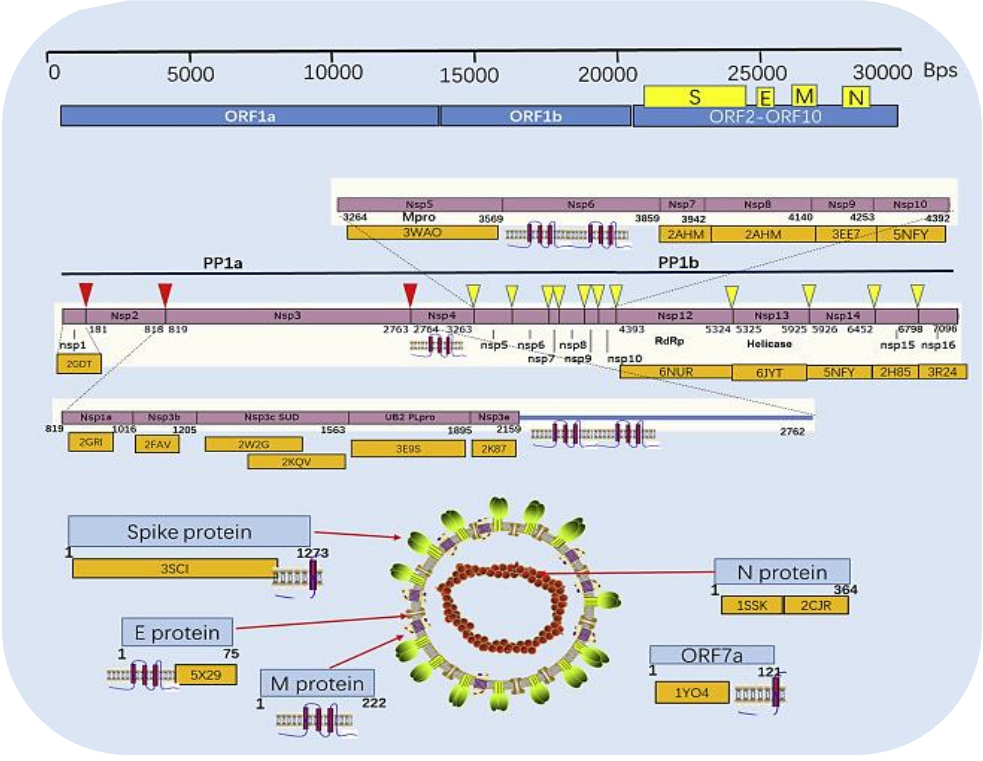


Figure 6. Overall Genome and Protein Analysis of SARS-CoV2 (Adopted from NCBI)



Each type of protein is associated to perform its specific function in the overall life of this virus [32].

Transmission of SARS-CoV2

SARS-CoV2 is transmitted under three varied conditions: viral infection source, transmission channel, and virus exposure [33,34].

Transmission by the Source of Infection

Bats are considered the natural source of SARS-CoV2. Pangolin and snakes are also thought to be the source of SARS-CoV2; however, later studies found no evidence indicating that snakes are a host of SARS-CoV2. According to a study, bat coronavirus and SARS-CoV2 have a 96.2% sequence similarity. Furthermore, pairwise protein sequence analysis of the seven conserved non-structural proteins' domain regions revealed that the virus belonged to the SARS-CoV species [34]. Additionally, the SARS-CoV2 originating from pangolin and the infected humans have 99% sequence similarity between them. This suggests that pangolin could be a SARS-CoV2 intermediate host.

Transmission by the Route of Transmission

COVID-19 is most commonly transmitted through close contact. It can potentially be transmitted through aerosols, urine, and saliva [35]. In silico studies revealed that the gastrointestinal system (GI tract) is a major path for SARS-CoV2 infection. This finding was also confirmed by identifying the constant presence of the virus in the GI tract of infected patients. Similarly, the tears and conjunctival secretions of COVID-19 patients also revealed the presence of the pathogen [36]. This virus also affects pregnant women; however, the available data on COVID-19-affected pregnant women is insufficient [37].

Transmission by the Viral Latency

Elderly people with a median age of 75 are more vulnerable to COVID-19. It was observed from the clinical characteristics of these patients that the virus has a 3 day incubation period and it takes 14 days from the onset of first symptoms to death [38]. SARS-CoV2 has a maximum latency of 24 days, which may enhance the likelihood of viral infection. It has also been discovered that those over the age of 70 have shorter median days; they have roughly 11.5 days from the onset of symptoms to death. People below 70 have 20 median days; this indicates a faster disease progression rate in elderly people than younger people [37].

By gaining detailed information about the genomic content of coronavirus, we can understand the structural as well as the non-structural composition of proteins, which, in turn, can help identify the routes of its transmission. Unfortunately, we are still far away from developing a suitable therapy because of the variation in the genomic organization of coronavirus.

Variations in SARS-CoV2 Genome

Current studies revealed the presence of variations in the SARS-CoV2 genome. This extrapolated the absence of an accessory protein ORF 8a as well as multiple changes in amino acids in ORF 8b and 3c proteins [39]. Moreover, the spike glycoprotein (S) of COVID-19 is altered through homologous recombination of SARS-CoV (bat) and Beta-CoV (unknown) [40]. To enter the host cell, SARS-CoV2 utilizes angiotensin-converting enzyme 2 (ACE2) cell receptors. A significant N501T mutation in the SARS-CoV S-protein was recently found to result in stronger binding with ACE2 receptors [6].

Lifecycle of Coronavirus

Coronaviruses go through the same stages as other viruses in their life cycle. These steps are attachment, replication, and release.

Attachment of SARS-CoV

ORF1 are the particular genes found in all of the coronaviruses. These genes code for the proteins involved in viral replication, nucleocapsid development, and spike creation. The spikes on coronaviruses' surfaces are responsible for the virus's adhesion and penetration into host cells. The receptor-binding domain (RBD) of the virus is connected loosely to host cells and causes infection in many hosts. SARS-CoV and MERS-CoV use the exo-peptidase receptor to gain access into human cells [36]. Other coronaviruses prefer to enter the host cell by recognizing aminopeptidases or carbohydrates as receptors [41].

Human proteases can break spike proteins and cause variations in penetration. The MERS-coronavirus receptor is dipeptidyl peptidase 4 (DPP4); whereas, the SARS-coronavirus receptor is ACE2. SARS-CoV2 has a conventional coronavirus structure composed of additional polyproteins, membrane proteins, and nucleoproteins [42]. The glutamine

residue at 394 positions in SARS-RBD CoV2's region interacts with the human ACE2 receptor's lysine 31 residue.

Entry to Host Cell

After receptor binding, the virus enters into the host cell's cytosol via S protein cleavage using cathepsin or TMPRRS2. Subsequently, the viral envelope fuses with the cellular membrane. This proteolytic cleavage happens at two locations within the S protein's S2 domain [41]. The first cleavage is important for dividing the S protein's fusion domains and RBD, where the other reveals the fusion peptide. A combination of these proteins occurs within the endosome of coronaviruses. Once the fusion peptide is fused to the membrane, the S2 heptad repeats are combined to form antiparallel helix bundles. Finally, it causes the viral DNA to be released into the cytoplasm [39].

Expression of Replicase Protein

The terminal polyproteins pp1a and pp1ab are produced by the production of two ORFs, namely rep1a and rep1b, which are expressed by translation of the replicase gene. The molecular weight of pp1a and pp1ab is 500kDa and 800kDa, respectively. By using a slippery sequence of 5'-UUUAAAC-3' and an RNA pseudoknot, the virus expresses these polyproteins involved in the ribosomal frame-shifting from rep1a to rep1b ORF [43]. Elongation of the ribosome is blocked by pseudoknot, which results in a change of the reading frame by reversing one nucleotide resulting in the formation of pp1ab [37].

Viruses use the phenomena of frame-shifting to control protein expression such as the ratio of rep1b:rep1a proteins. They postpone the synthesis of rep1b products until rep1a products have created a favourable environment for RNA replication. pp1ab and pp1a encompass the nsps 1-16 and nsps 1-11, respectively. Conversely, gamma coronaviruses do not comprise nsp1, since these polyproteins are divided into separate nsps. Coronaviruses consist of nsp3 and nsp5 genes that later synthesize papain-like proteases (PLpro and Mpro) or Serine type proteases, respectively, and are involved in cleaving replicase polyproteins [38]. Several nsps are combined into replicase and transcriptase complex to prepare RNA replication and transcriptional machinery [26].

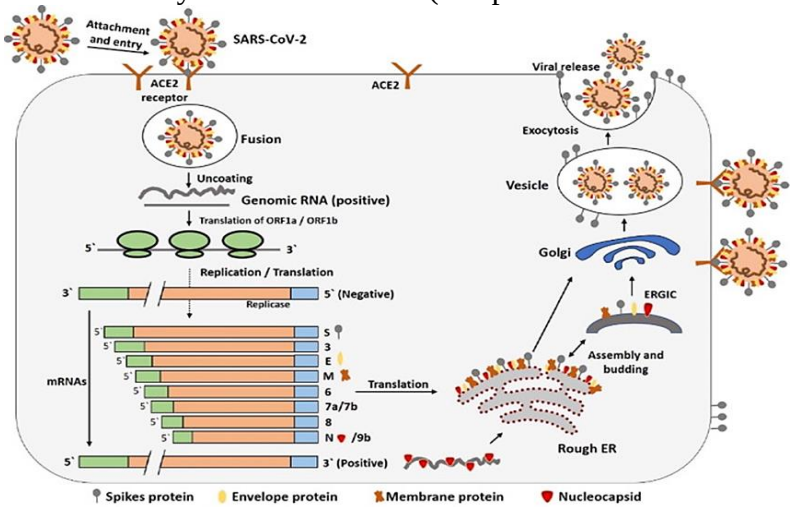
Replication and Transcription

Negative-strand intermediates are used to synthesize genomic and subgenomic RNAs. These intermediates comprise poly-uridylate as well as anti-leader sequences. The stem-loop structures present within the 5'UTR extends into replicase 1a. UTR at 3' end contains a pseudoknot, a protruded stem-loop, and a hypervariable region. Coronaviruses use homologous and non-homologous recombinations that play a crucial part in viral evolution [44].

Viral Assembly and Release

After replication and subgenomic RNA synthesis, the virus's structural proteins are expressed and integrated into the endoplasmic reticulum (ER). Alongside the secretory pathway, these proteins enter the ERGIC (endoplasmic reticulum-Golgi intermediate compartment). Encapsulated viral genome buds into ERGIC membranes comprising structural proteins to create mature viruses. The protein-protein interaction is directed by the M protein that is necessary for the virion assembly. Moreover, M and E proteins express and function together to fabricate envelopes of coronaviruses and form VLPs. Later, its formation is enhanced by the N protein [45]. At this step, S protein is integrated into the virion. M protein aggregation is prevented by the E protein, which changes the host secretory pathway and plays a key role in virion release [46].

Figure 7. The Life Cycle of Coronavirus (Adopted from Science Direct.com)



Next, M protein attaches to the viral nucleocapsid and aids in virion formation, while N protein aids in the packing of positive-sense genomes generated during infection. Virions are delivered to the cell surface as vesicles, which are then assembled and expelled via exocytosis. In coronaviruses, the S protein promotes cell-to-cell fusion between infected and host cells. This causes the virus to produce huge multinucleated cells, allowing it to spread throughout infected organisms without being recognized or killed by viral-specific antibodies [46]. The whole life cycle of coronavirus has been summarized in Figure 7.

Possible Ways of Prevention of COVID-19

COVID-19 has caused a lot of damage, which is why public health and infection control measures must be taken quickly to prevent the global spread of this virus [47]. More than 90 types of vaccines are being developed against SARS-CoV-2 in different universities and companies across the world[48].

COVID-19 is asymptomatic for some individuals, while for others it can cause flu-like symptoms, acute respiratory distress syndrome (ARDS), or pneumonia, both of which may lead to death. Although it is anticipated that an effective vaccine against COVID-19 will be available soon, for now, the world is relying on social distancing, hygiene measures, and repurposed drugs to prevent transmission of the virus. Even though, countries all over the world are trying to develop vaccines, however there are only 30 vaccines in clinical trials and over 200 in various stages of development. There is a worldwide effort to develop an effective vaccine against SARS-CoV-2, and by late August 2020, there were 30 vaccines in clinical trials with over 200 in various stages of development [49].

The development of an effective vaccine for COVID-19 is the urge of the whole world due to various complications and effecting daily life. About 30 vaccines are developed in the world to combat this life-threatening viral infection. Numerous trials were conducted to develop an applicable and reliable vaccine against COVID-19.

There are 3 phases in the development of vaccines. In the first phase, the safety of proper dose and monitoring complications are examined. In the second phase, the safety of the vaccine as well as its immunogenic

effect are analyzed. In the third phase, the efficacy of vaccines is calculated by comparing percentage of patients in which disease rate is reduced after receiving a placebo dose and the COVID-19 vaccine dose [49,50]. The best way to prevent the spread of this virus is by decreasing its transmission, which can be done by preventing mass gatherings, avoiding direct contact with patients affected with COVID-19, washing hands frequently, cleaning and disinfecting well-used surfaces with alcohol and bleach-based cleaning solutions, and using facemask [39].

Probable Treatment

Various drugs have been utilized for the treatment of COVID-19. Many of these drugs are still under clinical trial, while some are being practically used to treat SARS-CoV2 infected patients. Since the coronavirus that causes COVID-19 is related to the coronavirus that causes SARS and MERS, Remdesivir, an antiviral medicine, has attracted a lot of interest. Remdesivir, according to research, is a very effective medicine that prevents the virus from reproducing and spreading. Lopinavir and ritonavir is a medication combination used to treat HIV [51], it is now also being used to treat COVID-19. On February 18th, 2020, it was reported that an old woman with severe COVID-19-related pneumonia, was cured with this medicine combination [52]. Additionally, favipiravir, an antiviral medication used to treat influenza viruses, was approved for use in China on February 17th, 2020, and was used to treat new coronavirus pneumonia. Methylprednisolone is a glucocorticoid, it is also commonly used to treat new coronavirus infections. Furthermore, chloroquine phosphate is used to treat malaria and is identified to have multi-antiviral activity, which is being investigated for COVID-19 treatment. The drug hydroxychloroquine sulfate is used to treat lupus and arthritis in patients [53]. It is experimentally proven to be useful in the treatment of coronavirus pneumonia. A VEGF inhibitor, bevacizumab, is also being used to cure acute respiratory distress syndrome (ARDS) and acute lung injury (ALI). Azithromycin is an antibacterial drug, while hydroxychloroquine is an antimalarial drug [54,55]. It was first utilized in the treatment of 20 patients in France. According to the researchers, all of the patients who received the combination of these two medications were virologically healed within the 6-day therapy period [56]. Other drugs used to treat COVID-19 include Tylenol which reduces fever, chloroquine which

inhibits the attachment of viral S protein with ACE2 receptor, ritonavir which is a protease inhibitor that prevents the transition of viral proteins into their respective component, and corticosteroids which reduces inflammation by inhibiting arachidonic acid or phospholipase 2 [57].

Conclusion

Although the origins of COVID-19 and its transmission to human beings are unknown, human-to-human transmission is well-known due to its highly transmissible nature. Asymptomatic pyrexia, sore throat, dry cough, shortness of breath, body pains, lethargy, exhaustion, myalgia, vomiting, nausea, diarrhoea, and pneumonia are the common symptoms of COVID-19. These symptoms may cause acute respiratory distress syndrome (ARDS) and the dysfunction of various organs, leading to death. For this reason, COVID-19 has a case fatality rate of 2% to 3% [58].

Beyond vaccines, the global struggle against COVID-19 is a protracted one, until we produce effective and scientifically confirmed treatments. The first line of defence against this infection is preventing the spread of this disease. This review mainly examined all aspects of COVID-19 associated with its development into a pandemic. Overall, this review aimed to facilitate the prevention this disease as a pandemic disease before the innovation of any treatment and is also helpful in the development of possible treatment on understanding each aspect.

References

[1] Fehr AR, Perlman S. Coronaviruses: an overview of their replication and pathogenesis. *Coronaviruses*. 2015:1-23.

[2] Kahn JS, McIntosh K. History and recent advances in coronavirus discovery. *J Pediatr Infect Dis*. 2005;24(11):S223-S7. https://doi.org/10.1097/01.inf.0000188166.17324.60

[3] Rocklöv J, Sjödin H. High population densities catalyse the spread of COVID-19. *J Travel Med*. 2020;27(3):taaa038. https://doi.org/10.1093/jtm/taaa038

[4] Mousavizadeh L, Ghasemi S. Genotype and phenotype of COVID-19: Their roles in pathogenesis. *J Microbio, Immunol Infect*. 2021;54(2):159-63. https://doi.org/10.1016/j.jmii.2020.03.022

[5] Wrapp D, Wang N, Corbett KS, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Sci*. 2020;367(6483):1260-3. https://doi.org/10.1126/science.abb2507

[6] Shereen MA, Khan S, Kazmi A, Bashir N, Siddique R. COVID-19 infection: Origin, transmission, and characteristics of human coronaviruses. Journal of advanced research. 2020;24:91. https://doi.org/10.1016/j.jare.2020.03.005

[7] Sawicki SG. Coronavirus genome replication. Viral Genome Replication: Springer; 2009:25-39.

[8] Yadav A, Mohite S. Review on severe acute respiratory infection (SARI) and its clinical management in suspect/confirmed novel coronavirus (nCoV) cases Res. *J Pharma Dosage Forms and Tech.* 2020;12(3):178.

[9] Ba Abduallah MM, Hemida MG. Comparative analysis of the genome structure and organization of the Middle East respiratory syndrome coronavirus (MERS-CoV) 2012 to 2019 revealing evidence for virus strain barcoding, zoonotic transmission, and selection pressure. *Review Med Virol*. 2021;31(1):1-12. https://doi.org/10.1002/rmv.2150

[10] Ying H, Ebrahimi M, Keivan M, Khoshnam SE, Salahi S, Farzaneh M. miRNAs; a novel strategy for the treatment of COVID-19. *Cell Bio Int*. 2021;45(10):2045-53. https://doi.org/10.1002/cbin.11653

[11] Clark LK, Green TJ, Petit CM. Structure of nonstructural protein 1 from SARS-CoV-2. *J Virol*. 2021;95(4):e02019-20.

[12] Redondo N, Zaldívar-López S, Garrido JJ, Montoya M. Sars-cov-2 accessory proteins in viral pathogenesis: Knowns and unknowns. *Front Immunol*. 2021;12(2698). https://doi.org/10.3389/fimmu.2021.708264

[13] Yadav R, Chaudhary JK, Jain N, et al. Role of Structural and Non-Structural Proteins and Therapeutic Targets of SARS-CoV-2 for COVID-19. *Cell*. 2021;10(4):821. https://doi.org/10.3390/cells10040821

[14] Ali E, Raza MA, Cai M, et al. Calmodulin-binding transcription activator (CAMTA) genes family: Genome-wide survey and phylogenetic analysis in flax (Linum usitatissimum). *Plos One*. 2020;15(7):e0236454. https://doi.org/10.1371/journal.pone.0236454

[15] Beniac DR, Andonov A, Grudeski E, Booth TF. Architecture of the SARS coronavirus prefusion spike. *Nat Struct Mol Biol*. 2006;13(8):751-2. https://doi.org/10.1038/nsmb1123

[16] Abraham S, Kienzle TE, Lapps W, Brian DA. Deduced sequence of the bovine coronavirus spike protein and identification of the internal proteolytic cleavage site. *Virol*. 1990;176(1):296-301. https://doi.org/10.1016/0042-6822(90)90257-R

[17] De Groot RJ, Baker SC, Baric RS, et al. Commentary: Middle east respiratory syndrome coronavirus (mers-cov): announcement of the coronavirus study group. *J Virol*. 2013;87(14):7790-2.

[18] Armstrong J, Niemann H, Smeekens S, Rottier P, Warren G. Sequence and topology of a model intracellular membrane protein, E1 glycoprotein, from a coronavirus. *Nat*. 1984;308(5961):751-2. https://doi.org/10.1038/308751a0

[19] Nal B, Chan C, Kien F, et al. Differential maturation and subcellular localization of severe acute respiratory syndrome coronavirus surface proteins S, M and E. *J General Virol*. 2005;86(5):1423-34. https://doi.org/10.1099/vir.0.80671-0

[20] Nieto-Torres JL, Verdiá-Báguena C, Jimenez-Guardeño JM, et al. Severe acute respiratory syndrome coronavirus E protein transports calcium ions and activates the NLRP3 inflammasome. *Virol*. 2015;485:330-9. https://doi.org/10.1016/j.virol.2015.08.010

[21] Dinesh DC, Chalupska D, Silhan J, et al. Structural basis of RNA recognition by the SARS-CoV-2 nucleocapsid phosphoprotein. *Plos Pathog*. 2020;16(12):e1009100.

[22] 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.

[23] Sawicki SG, Sawicki DL, Younker D, et al. Functional and genetic analysis of coronavirus replicase-transcriptase proteins. *PLoS Pathog*. 2005;1(4):e39. https://doi.org/10.1371/journal.ppat.0010039

[24] Klausegger A, Strobl B, Regl G, Kaser A, Luytjes W, Vlasak R. Identification of a coronavirus hemagglutinin-esterase with a substrate specificity different from those of influenza C virus and bovine coronavirus. *J Virolog*. 1999;73(5):3737-43.

[25] Cornelissen L, Wierda C, Van Der Meer FJ, et al. Hemagglutinin-esterase, a novel structural protein of torovirus. *J Virol*. 1997;71(7):5277-86.

[26] Subissi L, Imbert I, Ferron F, et al. SARS-CoV ORF1b-encoded nonstructural proteins 12–16: replicative enzymes as antiviral targets. *Antivir Res.* 2014;101:122-30. https://doi.org/10.1016/j.antiviral.2013.11.006

[27] Littler DR, Gully BS, Colson RN, Rossjohn J. Crystal structure of the SARS-CoV-2 non-structural protein 9, Nsp9. *Iscience*. 2020;23(7):101258. https://doi.org/10.1016/j.isci.2020.101258

[28] Silva SJRd, Silva CTAd, Mendes RPG, Pena L. Role of nonstructural proteins in the pathogenesis of SARS-CoV-2. 2020. https://www.arca.fiocruz.br/handle/icict/40716

[29] Malik YA. Properties of coronavirus and SARS-CoV-2. *Malays J Patho*. 2020;42(1):3-11.

[30] Liu DX, Fung TS, Chong KK-L, Shukla A, Hilgenfeld R. Accessory proteins of SARS-CoV and other coronaviruses. *Antiviral Res*. 2014;109:97-109. https://doi.org/10.1016/j.antiviral.2014.06.013

[31] Narayanan K, Huang C, Makino S. SARS coronavirus accessory proteins. *Virus Res*. 2008;133(1):113-21.

[32] Brant AC, Tian W, Majerciak V, Yang W, Zheng Z-M. SARS-CoV-2: from its discovery to genome structure, transcription, and

replication. *Cell Biosci*. 2021;11(1):1-17. https://doi.org/10.1186/s13578-021-00643-z

[33] Lauritano D, Moreo G, Limongelli L, Nardone M, Carinci F. Environmental Disinfection Strategies to Prevent Indirect Transmission of SARS-CoV2 in Healthcare Settings. *Appl Sci*. 2020;10(18):6291. https://doi.org/10.3390/app10186291

[34] Reychler G, Vecellio L, Dubus J. Nebulization: A potential source of SARS-CoV-2 transmission. *Respiratory Med Res*. 2020;78:100778.

[35] Trypsteen W, Van Cleemput J, Snippenberg Wv, Gerlo S, Vandekerckhove L. On the whereabouts of SARS-CoV-2 in the human body: A systematic review. *PLoS Patho*. 2020;16(10):e1009037. https://doi.org/10.1371/journal.ppat.1009037

[36] Kaya H, Çalışkan A, Okul M, Sarı T, Akbudak İH. Detection of SARS-CoV-2 in the tears and conjunctival secretions of Coronavirus disease 2019 patients. *J Infect Develop Count.* 2020;14(09):977-81. https://doi.org/10.3855/jidc.13224

[37] Wang W, Tang J, Wei F. Updated understanding of the outbreak of 2019 novel coronavirus (2019-nCoV) in Wuhan, China. *J Med Virol*. 2020;92(4):441-7. https://doi.org/10.1002/jmv.25689

[38] Ji X-y, Ma Y, Shi N-n, et al. Clinical characteristics and treatment outcome of COVID-19 patients with stroke in China: A multicenter retrospective study. *Phytomedi*. 2021;81:153433. https://doi.org/10.1016/j.phymed.2020.153433

[39] Wu A, Peng Y, Huang B, et al. Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China. *Cell Host & Microbe.* 2020;27(3):325-8. https://doi.org/10.1016/j.chom.2020.02.001

[40] Li B, Si H-R, Zhu Y, et al. Discovery of bat coronaviruses through surveillance and probe capture-based next-generation sequencing. *Msphere*. 2020;5(1):e00807-19.

[41] Belouzard S, Millet JK, Licitra BN, Whittaker GR. Mechanisms of coronavirus cell entry mediated by the viral spike protein. *Virus*. 2012;4(6):1011-33. https://doi.org/10.3390/v4061011

[42] Lim YX, Ng YL, Tam JP, Liu DX. Human coronaviruses: a review of virus–host interactions. *Dis*. 2016;4(3):26.

[43] Graham RL, Sparks JS, Eckerle LD, Sims AC, Denison MR. SARS coronavirus replicase proteins in pathogenesis. *Virus Res*. 2008;133(1):88-100. https://doi.org/10.1016/j.virusres.2007.02.017

[44] Lau SK, Chan JF. Coronaviruses: emerging and re-emerging pathogens in humans and animals. *Virol J. 2015;*12:209-215. https://doi.org/10.1186/s12985-015-0432-z

[45] Ruch TR, Machamer CE. The coronavirus E protein: assembly and beyond. *Virus*. 2012;4(3):363-82. https://doi.org/10.3390/v4030363

[46] Contieri M, Trucillo M, Pavese L, et al. Can lifestyle have an impact on the incidence of COVID-19 disease? *Food Sci Report*. 2020;1:56-59.

[47] Caddy S. Developing a vaccine for covid-19. *BMJ*. 2020;369:m1790.

[48] Callaway E. The race for coronavirus vaccines: a graphical guide. *Nat*. 2020; 580(7805):576-7.

[49] Sharma O, Sultan AA, Ding H, Triggle CR. A Review of the Progress and Challenges of Developing a Vaccine for COVID-19. *Front Immunol*. 2020;11:2413. https://doi.org/10.3389/fimmu.2020.585354

[50] Singh K, Mehta S. The clinical development process for a novel preventive vaccine: An overview. Journal of postgraduate medicine. 2016;62(1):4.

[51] Doggrell SA. Does lopinavir measure up in the treatment of COVID-19? *Expert Opinion Investiga Drug*. 2020;29(8):793-6.

[52] Javorac D, Grahovac L, Manić L, et al. An overview of safety assessment of the medicines currently used in the treatment of COVID-19 disease. *Food Chem Toxicol*. 2020:111639. https://doi.org/10.1016/j.fct.2020.111639

[53] Cortegiani A, Ingoglia G, Ippolito M, Giarratano A, Einav S. A systematic review on the efficacy and safety of chloroquine for the

treatment of COVID-19. *J Critical Care*. 2020;57:279-83. https://doi.org/10.1016/j.jcrc.2020.03.005

[54] Oldenburg CE, Doan T. Azithromycin for severe COVID-19. *Lancet*. 2020;396(10256):936-7. https://doi.org/10.1016/S0140-6736(20)31863-8

[55] Echeverría-Esnal D, Martin-Ontiyuelo C, Navarrete-Rouco ME, et al. Azithromycin in the treatment of COVID-19: a review. *Expert Review of Anti-infective Therapy*. 2021;19(2):147-63. https://doi.org/10.1080/14787210.2020.1813024

[56] Zhang W, Zhao Y, Zhang F, et al. The use of anti-inflammatory drugs in the treatment of people with severe coronavirus disease 2019 (COVID-19): The Perspectives of clinical immunologists from China. *Clin Immunol*. 2020;214:108393. https://doi.org/10.1016/j.clim.2020.108393

[57] Orleans L, is Vice H, Manchikanti L. Expanded umbilical cord mesenchymal stem cells (UC-MSCs) as a therapeutic strategy in managing critically ill COVID-19 patients: the case for compassionate use. *Pain Physician*. 2020;23:E71-E83.

[58] Molintas DT. *Analysis of Coronavirus and carbon emissions.* MPRA Paper; 2020. https://mpra.ub.uni-muenchen.de/98858/