Journal of
Agriculture and Biology
ISSN: 3007-1763 (Online), 3007-1755 (Print)

Check for updates

ROOTS PRESS

Review Article

COVID-19: A deadly outbreak on planet earth

Muhammad Abubakkar Azmat[1], Rabia Rasheed[2], Saman Arshad[1], Muhammad Shaban[1], Amna Kanwal[3]

[1] *Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad (Burewala Campus), Pakistan.*
[2] *Department of Chemistry, University of Agriculture, Faisalabad (Burewala Campus), Pakistan.*
[3] *Fodder Research Sub-Station Ayub Agricultural Research Instititute, Faisalabad, Pakistan.*

ABSTRACT

Coronavirus may be a large group of viruses and recently identified coronavirus called SARS-CoV-2 or COVID-19 was first detected in Wuhan City, China. After that it spread rapidly to other countries. Some studies show positive, and few others exhibit negative relationship between COVID-19 and sunlight. There is controversy about its intermediate host. Snake, turtles, and pangolins are considered the intermediate hosts, however bat is regarded as the primary host. It belongs to Nidovirales order, family is Coronaviridae, and subfamily is Coronavirinae. The transmission may occur through various ways. The structure of COVID-19 is enveloped and spherical with a positive single strand. Various accessory, structural, and non-structural proteins are present in it. Life cycle involves attachment, entry, replication, translation, assembly, and then its release. B.1.1.7, B.1.351, p.1, and b.1.617 are a few important variants of SARS-CoV-2. An increase of cholesterol level supports the entry of SARS-CoV-2. Statins with lower cholesterol level lessens COVID-19 entry. Fever, cough, fatigue, headache, sputum production, diarrhea, dysphonia, etc. are common symptoms of COVID-19. RT-PCR, RT-LAMP, CT scan, antibodies and antigen are various tests for COVID-19 detection. Various drugs and convalescent plasma are used for its treatment. People are also now vaccinated, and these vaccines also have side effects like fever, fatigue, headache etc. Preventive measures must be carried out to prevent its spread.

Keywords: Covid-19, Vaccine, Structure of Covid-19, Causing Agent.

OPEN ACCESS

Correspondence
Muhammad Abubakar Azmat
abubakarpbg@uaf.edu.pk

Article History
Received: April 12, 2023
Accepted: June 13, 2023
Published: June 22, 2023

INTRODUCTION

A viral pandemic emerged in China during December 2019 (Lin et al., 2020). Later on, the research studies had confirmed that it is a Coronavirus. Coronavirus may cause disease in animals as well as in humans (Ahmad et al., 2020) like MERS and SARS. It causes sickness initiating with cold and leads to more severe diseases like acute respiratory infection (Keni et al., 2020). The word Coronavirus emerged from Corona, meaning "crown" in Latin. It possesses crown like spikes on its surface (Ganesh et al., 2021). Five human coronavirus epidemics had been occurred within the last twenty years (Prince et al., 2021). HCoV-NL63 secluded from a seven-month-old baby. It was first recognized in late 2004 in Netherlands. HCoV-HKU1 was first discovered in January 2004 in one male in Hong Kong (To et al., 2013). The more serious among these are MERS, SARS, and SARS-CoV-2 (Prince et al., 2021). MERS is a respiratory disease caused by MERS-CoV. First patient of MERS was identified in Saudi Arabia in 2012, originated in camels (Pillaiyar, 2015). Many cases were identified in Arabian Peninsula. SARS was first identified in February 2003 during an epidemic that emerged in China. The epidemic extended to other four countries. SARS is an airborne virus that originated in bats. The host was civet cat.

This novel coronavirus also called SARS-CoV-2 is a new virus, first identified in Wuhan city (Prince et al., 2021). SARS-CoV-2 spread all over the world and has affected humans in a very wide and complicated manner. Disturbance in national economies and businesses increased the unemployment rates, the travel industry has been badly disturbed, and the education system is also affected on a large scale.

Origin and spread to Pakistan

In December 2019, many pneumonia cases with unknown causing agent were reported in Wuhan. The first case was reported on 12th December 2019. While 27 viral pneumonia cases were officially announced on 31st December 2019 by Chinese officials. The research has shown the Huanan wholesale seafood market as the point of origin of Corona Virus that was also involved in the trade of live animals. On January 1st, 2020, the food market in Huanan Sea closed. On January 7, 2020, the virus's coronavirus identity was determined (Sahin et al., 2020). The potential for transmission from person to person expands throughout several Chinese provinces as well as other nations, and subsequently a pandemic breaks out worldwide. The pandemic spread was caused lockdowns regarding public movement, trade, and many other aspects of life in China and in all over the world later on. In start, Cases involving individuals returning from Wuhan were recorded. The main reasons for that spread include lack of knowledge of new viruses, insufficient detective tools, and non-implementation of accurate actions during the early time of outbreak. Reports of COVID-19 cases have also been made in nations other than China, particularly in those where there is no history of public travel to China and where there is evidence of local human-to-human transmission.

Many cases of COVID-19 were reported in Iran after China in Asia. Thousands of people from Pakistan visit Iran each year for religious and cultural purposes. On February 23rd, 2020, Pakistan closed its border with Iran. Hundreds of Pakistanis were barely managed to get back through Afghanistan or other means. In Pakistan first two cases were reported on February 26th, 2020. One case was reported in Karachi and the other in Islamabad. Both individuals have been returned from Iran. In order to prevent the spread of COVID-19, Government decided to quarantine people returning from Iran in Taftan city, a border town in Baluchistan. Two more cases were confirmed by the Government official on 29th February 2020. One case was reported from federal areas and the other from Karachi (Rasheed et al., 2020). Pakistan has been in a state of high alert afterward. The safety measures were later intensified with the rising number of cases and local spread to smart and complete lockdowns (Khan et al., 2021).

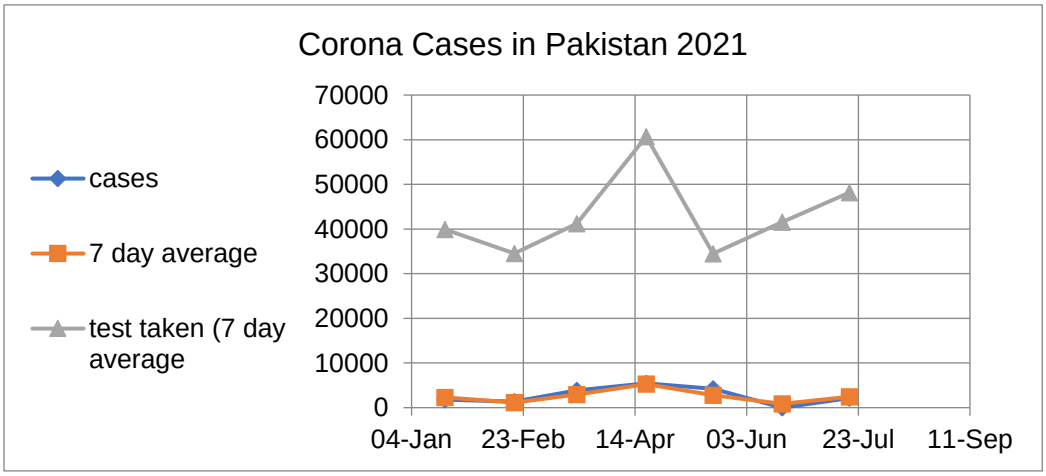

Figure 1. Corona cases in Pakistan: blue shows new cases, red shows 7 day average of cases and green shows 7 day average of test taken in year 2021.

Intermediate Host

Bats are the primary source of this virus (Guo et al., 2020). It is supposed that *R.affinis* and *R. malaynus* bats are natural hosts of SARS-CoV-2. As humans have no direct contact with bats, the direct transmission of CoVs from bats to humans is rare. The intermediate host that transfers the virus to humans is still unknown (Mahdy et al., 2020). Upon virus genome sequencing, the COVID-19 showed 96.2% sequence similarity with that of Bat CoV RaTG13 genome. Bats were not sold in the Wuhan animal market. Some studies showed that snakes are the main source of this virus. Researchers stated that potential intermediate hosts include other animals like turtles and pangolins (Guo et al., 2020). The genome of pangolin-CoV isolated from Malyan pangolin (*Manis javanica*) was similar to that of bat-CoV and SARS-CoV. There is a greater similarity between pangolin-CoV and SARS-CoV-2 than bat CoV. This suggests that the

pangolin may be an intermediate host and cross-species transmission occurs via this organism (Mahdy et al., 2020). A high similarity between pangolin virus and SARS-CoV-2 exists within that region of DNA which encodes for enveloped proteins. This similarity is 99%. While the full-length genome similarity is only 90.3% which is less as compared to similarity between RaTG13 and SARS-CoV-2. Pangolin CoV and Bat CoV RaTG13 do not carry the polybasic cleavage site insertion. The human ACE2 receptor requires this region. The SARS-CoV-2 virus is not the product of pangolin and bat viral recombination. Pangolins in China are nearly extinct, and no wild pangolin was caught. This low population seems to be not an intermediate host (Yuan et al., 2020). The RBD of the pangolin-CoV is similar to SARS-CoV-2. Thus, in the recombination of SARS-CoV-2, pangolin was involved. The SARS-CoV-2 did not arise directly from the pangolin-CoV, which was supported by phylogenetic analyses (Mahdy et al., 2020). Chinese were using pangolin skin scales as medicines in the ancient times. Malyan pangolins from Chinese city Guangdong, showed 85.5 to 92.4% genome sequence similarity to SARS-CoV-2.

The systemic comparison and the interaction divergence among the RBD and the ACE2 receptor showed that turtles such as Chrysemys*pictabellii*, *Cheloniamydas*, and *Pelodiscus* are potential intermediate hosts; however not confirmed, but snake might be an intermediate host. Studies also indicate frogs are more likely to be an intermediate host (Zhao et al., 2020). Therefore, scientists are still working to find the intermediate host.

Only one percent of the patients had direct experience with the live animal market trade, according to epidemiological studies. Over three-quarters of them were Wuhan locals. This demonstrates that viruses may spread from person to person (Wang et al., 2020). This virus was named Novel Coronavirus by WHO on 12th January and COVID-19 on 11th February 2020. Similar to the SARS epidemic, this outbreak was occurred during China's spring festival. During this period the SARS pandemic was at its peak. This significant traveling traffic had also produced favorable conditions for the spread of hard-to-manage disease within China (Sahin et al., 2020). Wuhan city is an international trade link that had play a role of pandemic spread in other countries.

Sunlight Inactivates SARS-CoV-2

Recent studies have shown that the COVID-19 virus is stable in cold environments. As temperature increases, the virus sensitivity is reduced. However, some reports from China, Brazil, the United States, and Turkey have been shown a negative association between the temperature and the cases reported. While in other countries, investigations have shown positive association among temperature and cases reported (Guo et al., 2021). A study shows that sunshine inactivates coronavirus 8 times faster. A study has found that the SARS-CoV-2 virus was 3 times more perceptive to the UV in sunlight than influenza. Generally, 90% of the coronavirus particles were inactivated after just an hour of exposure to midday sunlight in summer. According to the study, virus inactivation requires up to 20 minutes. It is much faster than formerly predicted by theory. Researchers developed a theory of coronavirus inactivation by solar radiation. The idea states that UV-B radiation interacts with the virus's RNA and causes damage to it, whereas UV-C light has been shown to be effective against SARS-CoV-2.

Classification

Corona viruses (CoVs) belong to the Nidovirales order. It is the largest group of viruses. The Coronaviridae forms two subfamilies i.e. Coronavirinae and Torovirinae. Coronavirinae is divided into four categories Alpha, Beta, Gamma and Delta coronaviruses (Sofi et al., 2020). Alpha and beta coronaviruses cause respiratory diseases in humans and gastrointestinal diseases in animals. Gamma and delta coronaviruses cause illness in birds mainly. These may infect mammals but never cause disease in animals and humans. Before December 2019, alpha and beta coronaviruses (6 coronaviruses) infect humans. HCoV-229E and HCoV-NL63 belong to the Alpha coronaviruses. HCoV-OC43 and HCoV-HKU1 belong to the lineage A of Beta coronaviruses. SARS-CoV and MERS-CoV belong to lineages B and C of Beta coronaviruses, respectively. SARS-CoV-2 genomic analysis shows that it belongs to the lineage B of Beta coronaviruses' group (Liu et al., 2020). The size, quantity, and form of the structural proteins are the primary distinctions amongst the Norovirus families. These variations result in significant modifications to the shape and structure of virions and nucleocapsids. The categorization was originally based on serology, but phylogenetic clustering now makes it stand out.

Transmission

The transmission of coronavirus can take place by the following modes.

Animal to Human Transmission of CORONAVIRUS

Coronavirus transmission routes to humans are not fully cleared; however, earlier, it was stated that it was transmitted to humans from bats. It shows that the infection is zoonotic (transmitted from animals (bat, pangolin, and snake) to humans). The virus is also transmitted in people having no interaction with animals, which is still unclear; however,

some reports also indicated airborne transmission, specifically cold places. Zhao et al., 2020, discovered the evidence of animal to human transmission via two farm workers which were infected from minks and stray cats in the surrounding of the farm (Zhao et al., 2020).

Human to the Animal Transmission of Coronavirus

SARS-CoV-2 can be transmitted to animals from humans. A few examples of transmission of COVID-19 from humans to animals are Minks that has been reported throughout a long history. They also farmed for their fur. In the Netherlands, SARS-CoV-2 attacked several mink farms, in fact, it was found that minks were dying and had respiratory symptoms. The virus was transmitted from a worker who had COVID-19 virus (Zhao et al., 2020). SARS-CoV-2 had showed infection in two dogs in Hong Kong. Both cases were reported in dogs having close interaction with SARS-CoV-2 positive owners (Tiwari et al., 2020). SARS-CoV-2 also infected cats in Wuhan city during COVID-19 outbreak. Cats got infected from the SARS-CoV-2 virus from their environment or their infected owners. Infection in two pet cats was reported in two separate places in New York. SARS-CoV-2 has been found in the vomit and feces of two sick pet cats that are housed with affected owners in Belgium and Hong Kong. The virus replicates in the upper respiratory tract of cats (Mahdy et al., 2020). The infection was appeared in the Malyan tiger at Bronx Zoo, New York. This was the initial instance of human-to-animal transfer. The tiger's sickness was thought to have come from its former owner. Poultry species like chickens, ducks, and geese are not susceptible to SARS-CoV-2. Cattles also show low susceptibility.

Human-to-Human Transmission

Human-to-human contact is the primary method of pandemic transmission. The patients of COVID-19 rapidly spread the disease to other people through close contact. Many who are asymptotic but carry the virus can spread the disease to others. The cases of secondary and tertiary transmission are confirmed. Thus, disease can spread rapidly from one generation to the next. The human to human transmission may occur in the following ways (Rahman et al., 2020).

Horizontal Transmission

There are three main routes of horizontal transmission. These are aerosols, direct contact, and droplets. The coronavirus is principally an airborne virus. Mostly, the coronavirus is spread through the air. As aerosols from expired air coughs and sneezes pollute the atmosphere, aerosol transmission may occur. Aerosol transmission is also possible from the asymptotic covid-19 positive people. The aerosol containing the virus can persist in the air for three hours. It remains for 48-72 hours on plastic surfaces and stainless steel. As a result, the concentration of virus increases, and it can spread more rapidly. Direct contact occurs when a person comes in contact with objects that are contaminated with viruses and can carry the virus. Droplet transmission occurs through air as the air in which we do respiration may contain an abundance of droplets less than 5micro meter in diameter. By coughing and sneezing, the droplets through the respiratory system may emit into the environment (Rahman et al., 2020).

Fecal Oral Transmission

The fecal-oral transmission is not yet clear; however, the gastrointestinal system is a route for this virus. It has been found that abundant expressions of ACE2 receptors in colon, ileum and rectal cells indicated the virus spread through feces (Rahman et al., 2020).

Transmission through Organ Transplantation and Surgical Instruments

Coronavirus remains in the respiratory tract and secretions. If the person with COVID-19 requires surgery, it poses a health risk to workers. Pulmonary and respiratory surgeries possess high risk (Rahman et al., 2020).

Vertical Transmission

The first case of vertical transmission of coronavirus was reported in March 2020. The pregnant women can highly be infected with the virus due to the abundance of expression of ACE2 in maternal fetal interphase (Rahman et al., 2020). The virus can cross the placenta, infect the fetus, and transmit via breast milk if the viral particle is smaller than the barrier of placenta. Since SARS-CoV-2 is larger and not cross and enter into breast milk, however, close contact may cause transmission of virus (Wiwanitkit, 2020).

Blood-Borne Transmission

There have not been any confirmed cases of viremia. In convalescent patients, viral particles can be detected for a long time. Since this is not confirmed that the viral particles in convalescent plasma can cause further infections or not. Further studies are required to confirm whether this transmission mode is feasible or not (Wiwanitkit, 2020).

Transmission via Skin Contact

SARS-CoV-2 is unlikely to transmit by contact with a wound or intact skin. However, the virus can transmit if the person’s hand comes in contact with skin surface with viral loading. Through contaminated hands, the virus can transmit to the body through mouth or nose (Wiwanitkit V, 2020).

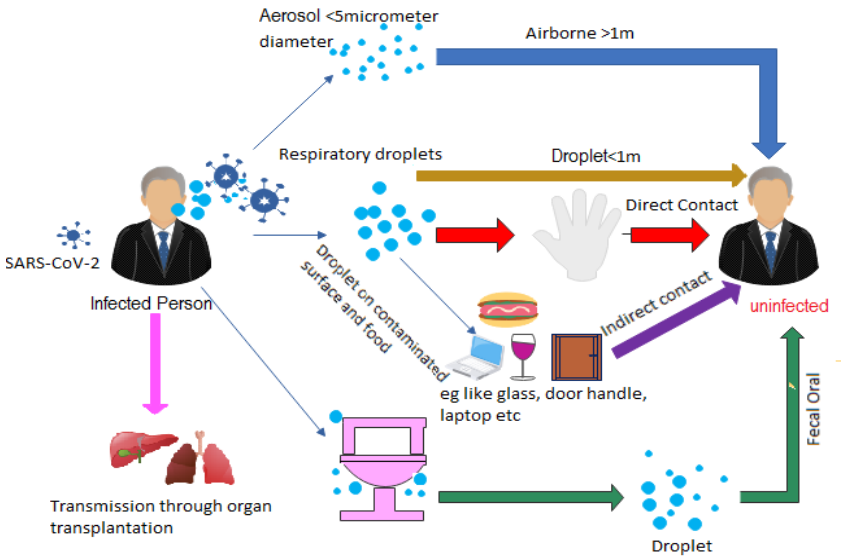

Figure 2. Transmission routes of COVID-19.

Structure and Replication of COVID-19

Structure

Covid-19 is a spherical enveloped virus possesses + ss RNA genome. This RNA is associated with a nucleoprotein however, hem agglutinin-esterase protein is present in some coronaviruses. The genomic size is between 27-32kbp. Contents of G+C varied from 32% to 43% (Mousavizadeh and Ghasemi, 2021). Five mutations have been recognized including T8782C which is a silent mutation that occurs in ORF1a, mutate codon AGT to AGC, and T9561C which is a non-silent mutation that occurs in ORF1a and mutate codon TTA to TCA. C15607T is also a silent mutation that occurs in ORF1b and changes codon CTA to TTA. C28144T, a non-silent mutation occurs in ORF8b that changes codon TCA to TTA. T29095C is a silent mutation that results in change of codon TTT to TTC and occurs in nucleotide side (Dagur et al., 2020). The structure of genomic RNA has 5'-cap and 3'-poly-A tail. The larger genome of Covid-19 is maintained due to special features of CoV RTC (Chen et al., 2020).

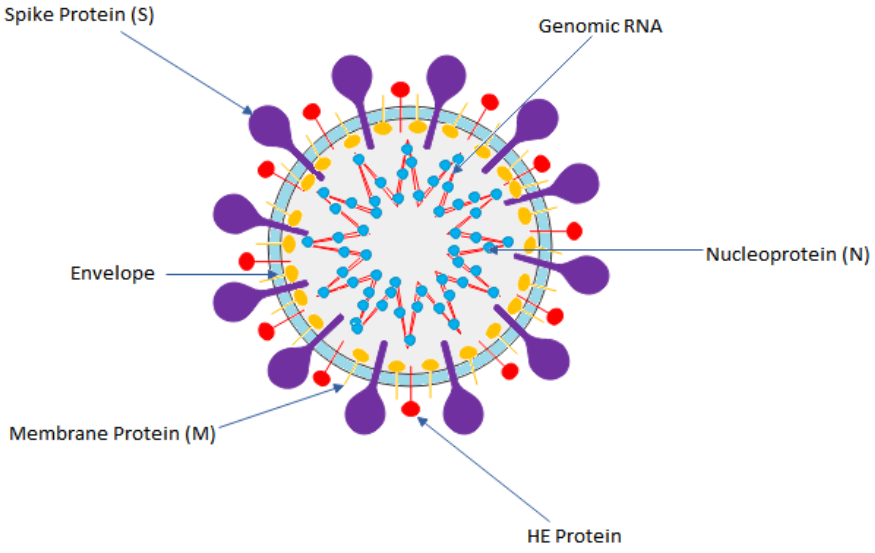

Figure 3. Structure of SARS-CoV-2.

Based on structural studies, sequence analysis shows that it has >80% similarity with SARS-CoV and 50% with MERS-CoV. The CoV-19 is more dangerous due to different epidemiological dynamics (Naqvi et al., 22020).

Secondary Structure of genomic RNA

Genomes of CoVs form secondary structures. These are the regulatory elements which are essential for a virus' life cycle. These play a role in virus replication. More variation had been seen in RNA structure. Quadruplet sites, inverted repetitions that create hairpins, and slippery sequences with downstream pseudoknots are examples of such SS. The host surveillance system is prevented from identifying the viral genome by a greater quantity of genome-scale organized RNA structures. The CoV-2 possesses the most IR frequency per thousand nucleotides. There is difference in the length of IRs which shows that these two untranslated regions have different regulatory roles. The 5' and 3' untranslated region contains IRs of length 12 plus and <12 nucleotides respectively. SL1-SL5 is five stem loop structure in the 5'-UTR regions. SL2 plays a role in sgRNA synthesis. SL5 has the start codon for ORF1a. ORF1ab was predicted to contain a pseudoknot structure. The programmed-1 ribosomal frameshift signal, which is produced when ORF1a and ORF1ab translate simultaneously, is involved in directing the frame shift. Additionally, the slippery sequence and linker region make up the 5'-1PRF signal. At 3'-UTR, there is an SL2-like motif. Crystal structure exists in SL2-like motif. It has an effect on how protein translation gets started. The guanine tetrad is a square planar structure formed from four guanine bases. The G-quadruplet is another SS formed by stacking the guanine tetrad (Kadam et al., 2021).

Virus Proteins and their Function

Various virus proteins and their functions are enlisted below.

Accessory Proteins

ORF3a, 3d, 6, 7a, 7b, 8, 9b, 14, and 10 are 9 accessory proteins produced from at least 5 ORFs (Yadav et al., 2021). Accessory proteins coding genes mainly clustered at the 3' end of the genome although these are present in between the structural genes that play a role in virus' life cycle and are considered to be replaceable. Few additional proteins are showed only selectively in a few CoVs. 3a, 6, 7a, 7b, 8, 9b are six accessory proteins encoding ORFs in CoV-2. SARS-CoV-2 lacks 8a and contains 8b. The accessory proteins have a role in counter attacking regarding host immune response (Kadam et al., 2021).

Table 1. Some Accessory Proteins

Accessory Proteins	Number of amino acids	Location	Description
ORF3a	274	Between S and E protein	• Accessory factor 3a is encoded by ORF3a • 3a protein is O-linked glycosylated and possesses three transmembrane domains • ORF3a forms a dimer • Ion channel in the host cell membrane is highly conductive for Ca^{2+} /K^{+} and is created by six transmembrane helices of ORF3a • In virus release, apoptosis, and pathogenesis, ORF3a is involved. (Yadav et al., 2021)
ORF3d	154	Nucleolus and mitochondria	• 3d protein is encoded by ORF3d. (Yadav et al., 2021)
ORF6	61	ER and Golgi compartments	• In virus infected Vector, E6 cells and in the lung, intestine tissue of patients' the expression of this protein was confirmed. (Yadav et al., 2021)
ORF7a	122	-	• Synthesized from bicistronic sub genomic RNA of SARS-CoV-2 • It is type-1 transmembrane protein that consists of 15 amino acids signal peptide sequence, 81 amino acids transmembrane domain and a short C-terminal tail. (Yadav et al., 2021)
ORF7b	44	Golgi compartment	• Synthesized from bicistronic sub genomic RNA of SARS-CoV-2 • ORF7b is an integral membrane protein

			• Expressed in SARS-CoV infected cells (Yadav et al., 2021)
ORF8	121	-	• Shows low homology to SARS-CoV • Shape is like Ig fold owing to beta strand core (18-121 residues), and interact with major histocompatibility complex-1 • It helps in immune evasion (Yadav et al., 2021)
ORF9b	97	-	• It suppresses IFN-1 mediated antiviral response by associating it with adaptor protein, TOM70. • ORF9b is dimeric (Arya et al., 2021)

Structural proteins

S protein

It is a type 1 transmembrane N-linked glycosylated protein. The molecular weight is about 150-200kDa and consists of 1273 amino acids (Yadav et al., 2021). The entry of CoVs into host cell is accompanied by engaging their S proteins with host cell receptors. Ectodomain region, transmembrane region, and intracellular domain are sites from which these proteins are composed. The S proteins can make hinge-like movement. The ectodomain has two subdomains, S1 acts as a major surface antigen. S1-CTD is a subunit of S1 which acts as a receptor binding domain that interacts with the 18 residues of ACE-2. S2 domain functions as a membrane fusion component in its basic form. Two cleavage sites are present. The HRs form coiled structure and facilitate fusion by taking virus envelope close to the host cell bilayer. A fur in cleavage site is present at the boundary of S1 and S2 subunits. This site distinguishes between SARS-CoV-2, SARS-CoV and other CoVs (Kadam et al., 2021). S1 determines host virus range. The receptor binding domain composition of it is also accountable for cellular tropism. In the spreading host cell, S2 mediates viral fusion (Astuti & Ysrafil, 2020). Mutation in SARS-CoV-2 is an adaptation. It increases the structural ability of S protein. It also weakens the attachment of antibody raised in opposition to other strains.

Membrane (M) protein

Membrane (M) protein is an O-linked glycoprotein of around 25-30kda and is much in CoVs and interacts with other structural proteins like N proteins. Coronavirus M protein undergoes N-linked glycosylation except beta and delta coronaviruses, which shows an O-linked glycosylation (Yadav et al., 2021). Three transmembrane domains characterize it. The amphipathic region at the end of the third transmembrane is present. This region is highly conserved among Coronaviridae members. The SARS-CoV-2 M protein shows mutation in N-terminal and transmembrane domain. It supports cross species transfer of the SARS-CoV-2. M proteins assist in viral assembly and its internal homeostasis. These also help control the process of RNA packing into mature virus particles and the regulation of replication. M proteins play a variety of activities, such as interacting with host cell NF-kB and contributing to pathogenesis by interfering with COX-2 and NF-kB-mediated host inflammatory responses (Kadam et al., 2021). Additionally, it collaborates with the S and E proteins to give the envelope its distinctive structure (Yadav et al., 2021).

Envelope (E) protein

It is a little polypeptide, weighing between 8 and 12 kDa. The architecture of the SARS-CoV-2 envelope protein displays a five-helix bundle. The amino acid sequences of E proteins differ greatly. These consist of a broad hydrophobic area, a short hydrophilic N terminus, and a hydrophilic C-terminal tail (Yadav et al., 2021). There are two domains in the envelope protein. These are the charged cytoplasmic tail and the hydrophobic transmembrane domain. It is a single spanning membrane protein. The C-terminus showing to the cytoplasmic side and N-terminus translocated across the membrane. The E protein shares the most common characteristic feature among CoVs. Oligomerization is formation of viroporin possessed by the E protein of COVID-19. The viroporins take part in the assembling and discharge of virus particles from host cells. Envelop protein assists in pathogenesis. Envelop protein helps in release of mature viruses by participating in the formation of ER-Golgi intermediate compartment (Kadam et al., 2021). The envelope protein is involved in the development and replication of viruses (Astuti & Ysrafil, 2020).

Nucleocapsid (N) protein

The CoV nucleocapsid is located in the Golgi and endoplasmic reticulum and is structurally linked to the virus's nucleic acid content (Astuti & Ysrafil, 2020). Three domains are separated among N proteins. These are N arm, central linker

or an RNA binding domain, and C tail. Important structural and functional domains are NTD and CTD. NTD plays role in RNA binding (Yadav et al., 2021). There are three areas in the NTD structure. These are specifically an acidic wrist, a basic palm, and an outstretched basic finger. The unique surface charge distribution of the SARS-CoV-2 NTD facilitates more effective binding to the RNA genome. CTD contains nuclear organization signal. N proteins perform a variety of tasks, including RNA transcription and replication, host-virus interactions, host cell control, and the creation and upkeep of the ribonucleoprotein complex. It also plays a role in viral protein assembling (Kadam et al., 2021).

Non-structural proteins (nsps)

NSP1 to NSP10 and NSP12 to NSP16 are non-structural proteins. NSPS plays various functions in SARS-CoVs.Nsp1 interacts with host mRNA and 40S ribosomal subunit and inhibits host protein synthesis. Nsp2 is N-terminal products. It binds to prohibiting 1 and 2 proteins and disturbs cell cycle. Nsp3 is papain like proteinase. In virus life cycle, it acts as a protease, involves in ssRNA binding, promote cytokine expression. In host pathogenesis, it hinders host translation by interacting with host RNA G-quadruplet &suppresses host immune response by ADPr binding. Nsp4 is membrane-spanning protein containing transmembrane domain2 that assists in assembling the viral double- membrane vesicles, viral replication transcription complex and modify ER membrane. Nsp5 is a proteinase and main proteinase. The role in virus life cycle is protease, inhibiting IFN signaling, cleaves at multiple distinct sites to synthesis mature and intermediate non-structural proteins. Nsp6 is putative transmembrane domains that induce auto phagosomes induction and DMV formation. Nsp7 is RNA-dependent RNA polymerase. It is cofactor with nsp8 and nsp12, in virus life cycle involve in RNA replication and primer synthesis.

Nsp8 is multimeric RNA polymerase; replicase. Role in virus life cycle, it is cofactor with nsp7 and nsp12, RNA replication and primer synthesis. Nsp9 is ssRNA-binding viral protein. In virus life cycle it has putative role as ssRNA binding, dimerization. In host pathogenesis, facilitate virus replication by interacting with DEAD-box RNA helicase 5 cellular protein. Nsp10 is a Growth-factor-like protein holding two zinc bound motifs and in virus life cycle involve in methylation of mRNA cap, & scaffold protein for nsp14 and nsp16. Nsp11 includes the same 13 amino acids as the first segment of Nsp12. Role in virus life cycle is not known. Nsp12 is RNA-dependent RNA polymerase involve in RNA replication, mRNA capping, &primer dependent RdRp in virus life cycle. Nsp13 is RNA-dependent RNA polymerase (Pol/RdRp). During RNA replication, helicase activity and 5' triphosphatase activity for mRNA capping. Nsp14 is Proofreading Exoribonuclease domain (ExoN/nsp14). Role in virus life cycle is N7 methyltransferase during mRNA capping, proof reading during RNA synthesis, exoribonuclease. Nsp15 is EndoRNAse; nsp15-A1 and nsp15B-NendoU. Endogonuclease cleaves RNA at polyuridylate sites, which plays a role in the life cycle of viruses. It is 2′-O-ribose methyltransferases, or Nsp16. During mRNA capping in the viral life cycle, 2'-O-ribose methyltransferase is involved.

Life cycle

The life cycle of SARS-CoV-2 involves the following stages

Virus Attachment and Entry

The virus uses its spike proteins to attach to certain receptors (ACE2) in order to enter the cell. Cellular receptors for CoV-19 include ACE2 receptors expressed on intestinal and lung epithelial cells, as well as, to a lesser degree, in the kidney, fat, heart, and both male and female reproductive tissue. Next, by proteolytic cleavage, a host protease activates the spike protein. The recently released N-terminus of the S2 domain is inserted into the cell membrane. Viral RNA is transferred into the host cell's cytoplasm when the viral and cellular membranes fuse. Coronavirus enters host cell through one of two distinct pathways. One is the cell surface pathway, and the other is the endocytic pathway.

Following serine proteases like TMPRSS2, the cell surface pathway was activated. Processing by lysosomal catharsis is part of the endocytic route, which is located within the endosomal-lysosomal gaps. The expression of protease, especially TMPRSS2, largely determine the pathway for entry. In the absence of protease, the endocytic pathway and activation by catharsis L is followed for entry. When TMPRSS2 or some other serine protease is expressed than the cell surface pathway is followed. (Murgholo et al., 2021).

Translation and Replication

The replication gene is translated from the virion geomic RNA once the viral genome has been released into the cytoplasm. The genomic material of virus is mRNA and it is + single strande. After entry into cell, by a frameshift mechanism, ORF1a and ORF1b are translated. The rep1a and rep1b are two large ORFs encoded by replicase gene. Rep1a and rep1b express two coterminal polyproteins means pp1a and pp1ab. During coronavirus infection, the genome is replicated and mRNAs are transcriptionally produced. Replication is the process which involves the synthesis - sRNA. It is a template for full length genomic RNA (Malik et al., 2020).

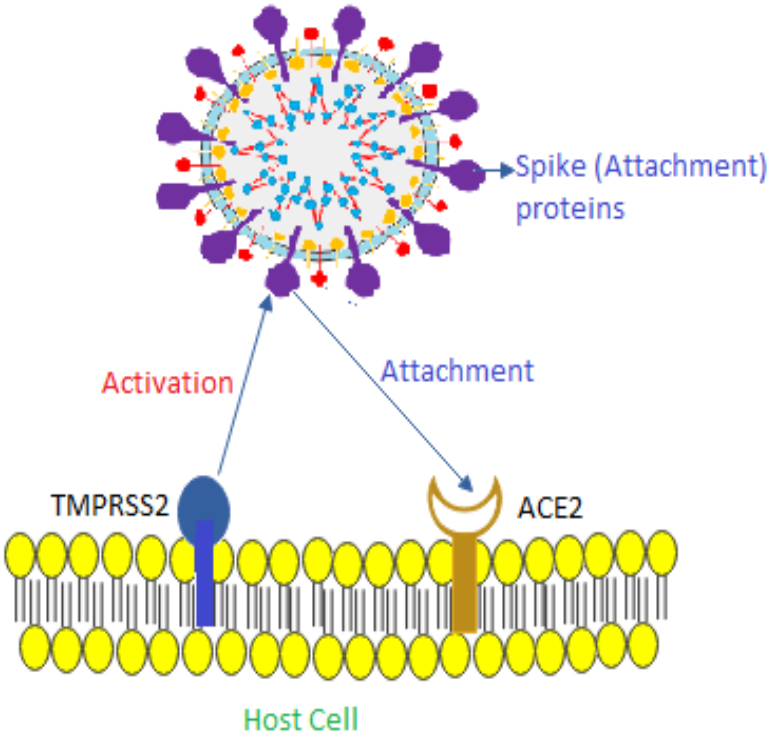

Figure 4. Attachement of SARS-CoV-2.

Polyproteins pp1a and pp1ab are proteolysed into smaller proteins by virus encoded proteinases. These proteins are involved in replication and transcription. Replication is facilitated by smaller proteins combining with the genomic RNA sense strand. Genomic RNA (-) is produced when the coronavirus's + strand RNA is reproduced. The RNA (-) either replicates to produce additional RNA (+) or uses discontinuous transcription to produce subgenomic mRNAs from the RNA(-) and numerous start sites in various ORFs. A discontinuous transcription mechanism is a mechanism by which group of (+) and (-) stranded RNA is syntheized. During the synthesis of sub genomic negative stranded RNAs, discontinuous transcription occurs when the antileader sequence is added to the 3' ends of negative strand RNAs. For synthesis of mRNAs, these negative stranded RNAs function as templates. From the 5' end of the genomic RNA, the replicase is translated. The subgenomic mRNAs are translated into (structural) S, E, M, N proteins (Liu et al., 2020).

Assembly and release

M, S, and E proteins are inserted in ER membrane after translation. After that, these are transported, through a secretory pathway, to the endoplasmic reticulum Golgi intermediate compartment which is considered to be the site of assembly. Assembly requires intricate coordination between M and other structural proteins, particularly E protein. In the cytoplasm, a nucleoprotein complex forms with freshly synthesized genomic RNA to create NP. the endoplasmic reticulum is reached by NP Golgi intermediate compartment, whereby NP condenses with envelope components to produce progeny viruses. Finally, viral exocytosis takes place and it ready to infect other cells (Lebeau et al., 2020). Lysosomal exocytosis pathway for release is the most recent one (Murgholo et al., 2021).

Figure 5 describes entry of SARS-CoV-2 by binding to ACE2 receptors via spike proteins. RNA from the virus is released into the cytosol. The polyproteins pp1a and pp1b are created when RNA is translated, and these proteins then break down into smaller ones. Ribosomal frame shifting occurs during SARS-CoV-2 replication. Genes related to the relevant viral proteins are produced by disontinuous transcription of both genomic and multiple copies of subgenomic RNA. The interaction of viral protein and RNA at the golgi complex and ER leads to virion assembly. Vesicles are used to discharge the virions outside the cells by exocytosis (Kumar et al., 2020).

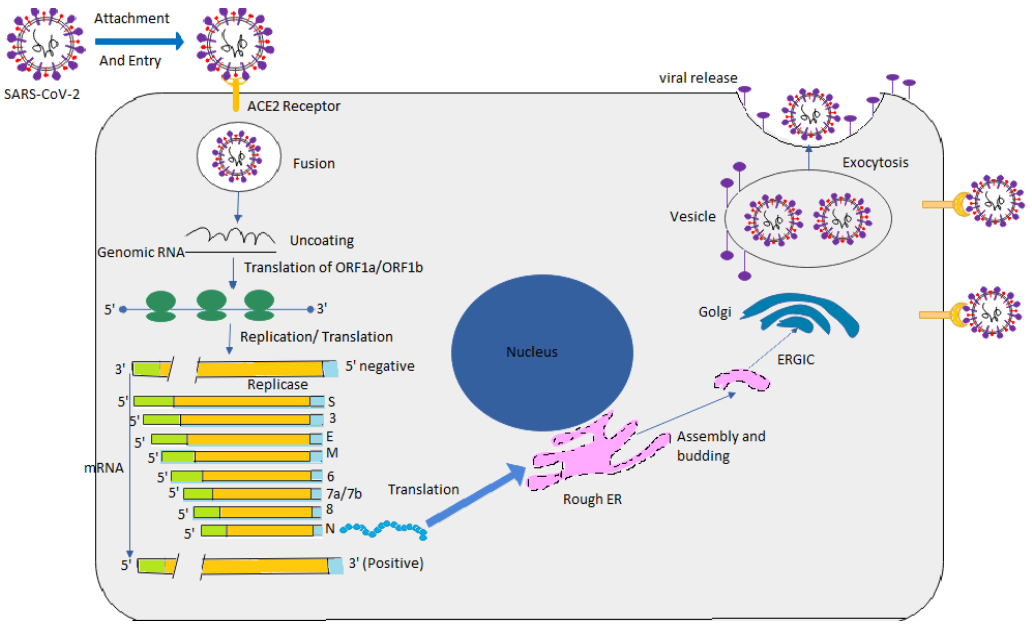

Figure 5. Life cycle of Covid-19.

Variants

B.1.1.7 lineage was first detected in late December 2020 in the Colorado, UK (Walensky et al., 2021). It is the most transmissible and subsequently spread to other countries. Nine mutations in the spike proteins have been identified in this lineage; six of them are situated in the S protein's surface unit, and three are in the transmembrane unit, S2. Exchange N501Y is situated in the RBD domain, and there is a correlation between it with a higher risk of human-to-human transmission (Hoffmann et al., 2021). B.1.351 lineage was first detected in the Republic of South Africa in December 2020. It is also known as 20H/501Y.V2. In late January 2021, it was identified in South Carolina and in Maryland. P.1 lineage is also known as 20J/501Y.V3. It was first detected in travelers from Brazil in December 2020 (Walensky et al., 2021).

B.1.617.1 and B.1.617.2 were first identified in December 2020. Each of sub lineage have spike protein mutation that have been associated with increased transmissibility. This variant is more dangerous. Although B.1.617.1 has been dubbed the "Indian double mutant," this label is deceptive because it differs from prior versions by about 15 mutations. Double mutant refers to the presence of two mutations in the virus's outer spike protein, L452R and E484Q. The term Indian Variant was later used as it was first detected in India. But it was contrary to the WHO policies that discourage the use of names that stigmatize countries. B.1.617.2 lacks E484Q mutation but has other mutations that are not existing in B.1.617.1. These mutations make the existing vaccines less effective.

This variant is more transmissible than previous ones. B.1.617.2 was recognized by the British Scientist at Public Health England on 7 May 2021 as variant of concern since it spreads more quickly. The variant spread to other countries. But in some countries due to lack of specialized kits for the variant detection, the variant detection was hindered. B. 1. 617 genomes have 13(15, or 17) mutations. Position 614 is where aspartic acid is substituted with glycine (D614G). At position 484, glutamic acid is substituted with glutamine (E484Q). This suggests a stronger binding potential of B. 1. 617 to the hACE2 and better ability to evade host immune system than other variants. Position 452 has a leucine to arginine alteration, designated as L452R. This demonstrates that spike proteins have a greater affinity for ACE2 receptors and a reduced capacity to recognize immune cells. P681R is the replacement of arginine for proline at position 681. By doing this, the S precursor protein is more easily cleaved into the active S1/S2 structure.

Role of Cholesterol

Lipid rafts are enriched in cholesterol and glycosphinogolipids and are plasma membrane subdomains. These are crucial for the entrance of viruses into host cells. The substantial abundance of cholesterol inside lipid rafts plays a crucial function in boosting viral infectivity. Lipid rafts play role in the interaction between S protein and ACE2 receptors and facilitate the process of viral endocytosis. Caveolins, clathrins, and dynamin also present in lipid rafts which also play role in virus entry like cholesterol. Hence cholesterol promote viral entry and replication.

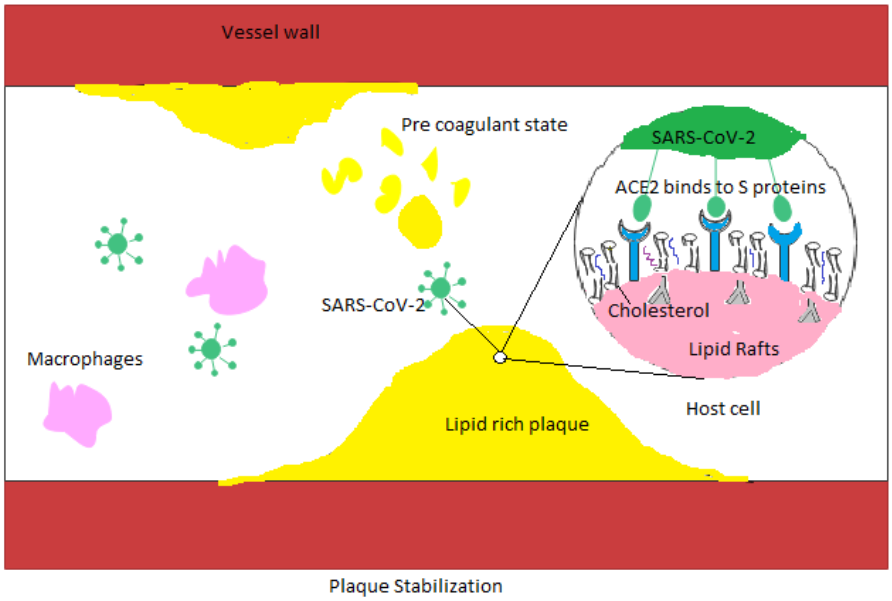

Figure 6. Mechanism of role of cholesterol.

Figure (6) shows that lipid rafts rich in cholesterol serve as sites for ACE2 receptor and viral attachement by the S protein of COVID-19. Clathrin takes it into the cell. Plaque volatility and emobilization brought on by an acute SARS-CoV-2 and macrophage infection obstruct the distal microvasculature (Radenkovic., 2020).
Drugs that change cholesterol have antiviral properties that reduce the production of cholesterol, reduce its absorption into the bloodstream, or directly affect the cholesterol in the target cell membran. Therefore, improve HDL functionality, increasing Apo-A1 levels by pharmacotherapy, blocking scavenger receptors by using neutralizing antibodies, controlling raised level of eicosanoids and hyper coagulation by combined therapy with omega-3 fatty acids and aspirin, and manage cytokine storm by the supplementation of eicosapentaenoic acid and docosahexaenoic acid are effective in COVID-19 patients. (Garg and Khanna, 2021)

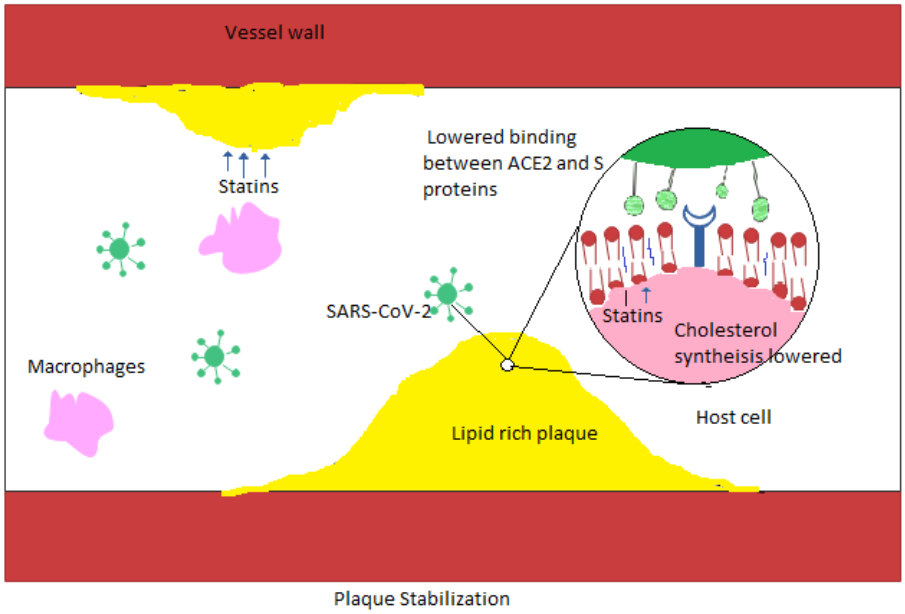

Figure 7. Stations in COVID-19.

Epidemiology Of COVID-19

Symptoms

The SARS-CoV-2 infection symptoms manifested themselves following a 5.2-day incubation period. The initial signs of a COVID-19 infection are fever, exhaustion, and cough. Sputum production, hemoptysis, headache, dysphonia,

diarrhea, and lymphopenia are later symptoms. A CT scan of the chest earlier identified pneumonia. The following abnormalities contributed to death: RNAaemia, acute respiratory syndrome, acute heart damage, and incidence of grand-glass opacities (Rothan and Byrareddy, 2020). The binding of S protein initiates the infection of SARS-CoV-2 to ACE2 receptors. ACE2 is highly expressed in the pulmonary epithelial cells, vascular epithelial cells, and cardiac monocytes. This led to extensive cardiopulmonary symptoms. Angiotensin 1, 2 convert to cardio protective peptides, angiotensin 1-9 and angiotensin 1-7 by ACE2. It loses on cell surface. Potential cardiac damage occurs. The loss of ACE2 on vascular endothelium may cause endothelial dysfunction, thrombosis, and inflammation. Electrical and mechanical dysfunctions are cardiac complications of COVID-19 (Sattar et al., 2020). Various symptoms are associated with various organ systems (Nalbandian et al., 2021)**.** Fever (98%), cough (76%), and fatigue (44%) were the most common symptoms in the initial 41 patients. Sputum production (28%), headache (8%), hemoptysis (5%), and diarrhea (3%) were fewer common symptoms. Dyspnea was developed in more than half of patients. Leukocytes (25%) and lymphopenia (65%) were reduced (Jin et al., 2020).

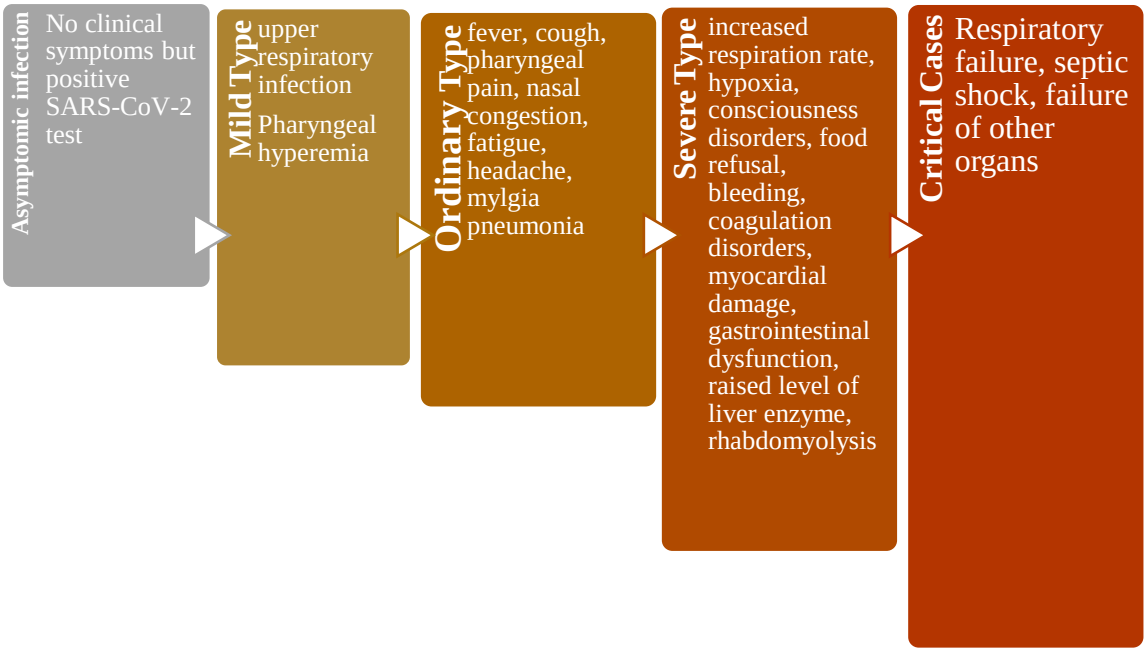

Figure 8. COVID-19 symptoms from mild to critical cases

Diagnosis

The viral shedding peaks at 5 to 6 days in throat and swabs after symptoms onset. In infected people's nasal swabs, the viral RNA detection rate has come up to 100%. The detection rates in blood, saliva, and tears are 88, 78, and 16% respectively. Some tests used for diagnosis of SARS-CoV-2 are given below

RT-PCR

The gold standard is RT-PCR for the identification of viral nucleic acids. Patient samples are obtained by nasopharyngeal swabs. SARS-CoV-2-infected cells and free virus particles are present in the fluid from which the RNA is isolated. Next, reverse transcription of the recovered viral RNA to cDNA is performed. Viral nucleic acid detection uses an amplified version of it. A florigenic probe is used in qPCR to amplify the subgenomic viral portions of the RdRp and E genes that are conserved. The detection threshold increased in positive cases (Kevadiya et al., 2021). Viral RNA detection by RT-PCR can achieve a 30-60% sensitivity. A negative nucleic acid test may result if patients with mild disease have a low viral load in nasal and pharyngeal swabs. But it is not clear that the viral load positively correlates with severe symptoms. The ultimate detection effectiveness of SARS-CoV-2 is affected by its low stability and ease of degradation by RNA enzymes released upon external destruction. False negative findings from the nucleic acid kit were caused by inadequate sampling strength, inappropriate sampling site, and inconsistent sample delivery mechanism. In order to improve results of RT-PCR multiple samples must be collected at various times must be collected from patients. Regardless of gastrointestinal symptoms, select fecal samples for nucleic acid test may be a better strategy (Wang et al., 2020).

RT loop-mediated isothermal amplification (RT-LAMP)

It is a quick, one-step method of amplifying DNA. Its benefits include rapid detection and minimal reliance on complex equipment. The reaction occurs at a temperature of 60-65^0 C in less than one hour. Because of RT-LAMP's single response, detection may happen quickly and reaction times are reduced. Real-time visual turbidity detection of the findings is possible. Consequently, the outcomes are visible to the unaided eye. Healthcare workers easily master this skill because the procedure is simple (Subail & Wiyono., 2020). Sensitivity, specificity, robustness, and cost are its advantages. In a single step, RT-LAMP amplifies DNA from an RNA target by combining LAMP. It immediately adds to the reaction mixture a specific reverse transcriptase or a DNA polymerase that exhibits reverse transcriptase activity. RT-LAMP mainly targets ORF1ab and gene N sequence. Some studies also target gene S, gene E, and gene M sequence (Diego et al., 2021).

The basis of RT-LAMP is paper that is incorporated into a microfluidic platform. It offers a viral diagnostic using a lab-on-a-chip. Fluorescein, the result of the reaction catalyzed by labeled RT, is attributed to a single primer set in the test. An other technique to produce a visible violet hue and allow for the identification of 100 copies per reaction is the use of leucocrystal violet dye. RT-RPA and RT-LAMP are combined in a single tube to create a closed tube Penn-RAMP, which raises the detection limit. CoV-RT-LAMP technique do not require extraction of RNA. The results have 72.7% sensitivity (Wang et al., 2020). LAMP technique can detect COVID-19 in saliva. This identifies infections in the salivary glands and releases certain biomarkers into the mouth cavity. One particular test tube is used to collect saliva. It was subjected to quick lateral flow experiments after being tagged with a particular biomarker protein.

Computed Tomography Scan

It is most likely faster, more helpful, and more dependable technology for COVID-19 diagnosis. Because CT image screening is available in nearly all hospitals, this can be utilized for early COVID-19 identification. However, thorax CT requires a radiology expert, and precious time is lost. Ground glass opacities, consolidation, crazy paving pattern and reticular pattern are usually visualized by CT scan for the diagnosis of COVID-19 (Shah et al., 2021).

Antibody Tests

Antibodies are proteins produced by the immune system to respond to viral attack. Antibodies tests identify the presence of antibodies that have been developed as a part of immune response (Zhang et al., 2020). For the quick detection of COVID-19 antibodies or anti-genes (spike, membrane, and nucleocapsid proteins), use the antibody test. Antibody testing's uneven ability to identify tiny amounts. Antibody tests can be useful after 2 weeks of infection. It is not known if it could be applied after a month's exposure.

Table 2. Antibody test types.

Type of test	Time to results	Antibodies
Chemiluminescent immunoassays	1-2 hours	IgG and IgM, & IgA
Neutralization assay	3-5 days	N/A
Enzyme-linked immunosorbescent assay	2-5 hours	IgG and IgM
Rapid diagnostics test	10-30 minutes	IgG and IgM

The procedure involves coating the plate with a protein specific to the virus and loading it with a sample of blood serum. If antibodies are present and attach to the viral antigen, an antigen antibody complex is created. On this plate, secondary antibodies tagged with fluorochrome, or substrates were added to identify the generated antigen-antibody complex. A detectable color change is produced (Arsani et al 2020). In the test, blood samples are obtained during the first week of illness. After three to four weeks, blood samples are taken once more in order to detect SARS-CoV-2 antibodies. After five days of infection, the igM positive rate shot up to 81%. igG positive rate in COVID-19 patients rose from 81% to 100% (Kopel et al., 2021). IgM level increased firstly followed by development of IgG. IgG against SARS-CoV-2 was already present in blood at high levels at the same time as, or before, IgM. IgG and IgM specific to SARS-CoV-2 peaked 17–19 days and 20–22 days following the beginning of symptoms, respectively (Azkur et al., 2020).

Antigen tests

In this test, an antigen in the sample binds to antibodies attached to a paper strip that is housed in a plastic containe. This reaction within half an hour generates a visual detectable signal. If the virus is actively replicating, the detected antigens are expressed. This test can be used to detect early infection.

Treatment

The patients who have asymptomatic, mild infection do not require special treatment. They only need to be self-quarantine. The general treatment includes bed rest, monitoring vital signals, ensuring sufficient calories, maintain homeostasis, and maintain an unobstructed respiratory tract. Patients with hypoxemia (SpO_2 < 95%) should receive oxygen therapy (Miao et al., 2020).

Remdesivir

It is a nucleotide analog (Tannak et al., 2020). Most promising of all is Remdesivir. It stops RNA-dependent RNA polymerase from working. As a result, viral RNA is prematurely inhibited. It puts an end to the viral genome's replication. This antiviral drug exhibit antiviral activities against SARS, MERS, in vitro, and in vivo. It has clinical trials for COVID-19 (Tu et al., 2020).

Favipiravir

In February 2020, it was studied for experimental treatment of COVID-19. It is a purine base analog. Error-prone viral RdRp incorporates it into the developing viral RNA. Viral mutagenesis and chain termination result from this. Favipiravir-RTP incorporates an RNA virus and then acts as a mutagen. It has the ability to escape from coronavirus repair equipment. Pressure on the CoV nucleotide content is increased by favipiravir-RTP. It not only increases the frequency of mutations but also benefits SARS-CoV-2 by reducing the number of infectious particles and viral RNA and having a cytopathic impact. Favipiravir target RdRp of COVID-19 (Joshi et al., 2021).

Lopinavir/ Ritonavir

COVID encodes cysteine protease. Theoretical evidence suggests that Lopinavir/ ritonavir inhibits $3CL1^{pro}$ protease of coronavirus. It has little or no benefit in COVID-19 patients (Tu et al., 2020). It inhibits coronavirus protease activity in vitro. It was initially seen as a treatment for COVID-19 because of its effectiveness in SARS and MERS. A recent randomized controlled trial shows no definite benefit of LPV (Parasher, 2020).

Chloroquine and Hydroxyl-Chloroquine

Chloroquine increases the endosomal pH required for virus/cell fusion and blocks virus infection. Hydroxyl chloroquine is more effective than chloroquine. Both these drugs reduce immunological response (Zhai et al., 2020).

Convalescent Plasma

The immunological plasma obtained from people who have recovered from infectious illnesses is known as convalescent plasma. If vulnerable individuals get this plasma transfusion, they may have short-term, instantaneous passive immunity. COVID-19 patients may benefit from convalescent plasma therapy. It reduces viral load and improves survival (Chen et al., 2020).

Vaccines

Active immunity against a particular microbial illness is produced by a vaccination. Vaccines' main purpose is to direct and prime the immune system to identify and combat certain viruses and bacteria (Salahshoori et al., 2021). Adjuvants, antibodies, stabilizers, preservatives and trace components are various components of vaccines. RNA, DNA, recombinant protein, & viral vector based are various types of vaccines that target S protein and live attenuated, recombinant vaccines target whole Viron of SARS-CoV-2 (Amanat & Krammer., 2020).

Side Effects of COVID-19 Vaccine

The WHO reports that COVID-19 vaccinations may have mild to moderate adverse effects. Injection site discomfort, fever, exhaustion, headaches, muscular aches, chills, and diarrhea are common adverse effects. Although rare, severe allergic responses including anaphylaxis are less prevalent adverse effects. After immunization, experiencing side effects is a sign that the shot is effective, and your immune system is responding. Nonetheless, vaccinations guard against COVID-19 and are safe (Murgolo et al., 2021).

Prevention

Prevention is better than cure. Some CDC recommendations are to wash hands with soap and water for at least 20s before preparing or eating food, after using the toilet, when coming from public places, after coughing, blowing your nose, and sneezing. Don’t touch your nose, mouth, eyes because these are the direct pathway for virus entry. If you are at home, keep 6 feet distance and prevent close contact with sick person. Always wear mask to cover mouth and nose in face of others. Avoid going in crowds like restaurants, bars, fitness centers, or movie theaters. The frequently touched surfaces like tables, toilets, sinks, door buttons, telephones, light switches, handles, keyboards, etc., must be cleaned regularly.COVID-19 symptoms must be checked regularly. Stay home stay safe. If symptoms like fever, cough, shortness of breath etc, seek medical attention. Self-quarantine to you (Salahshoori et al., 2021).

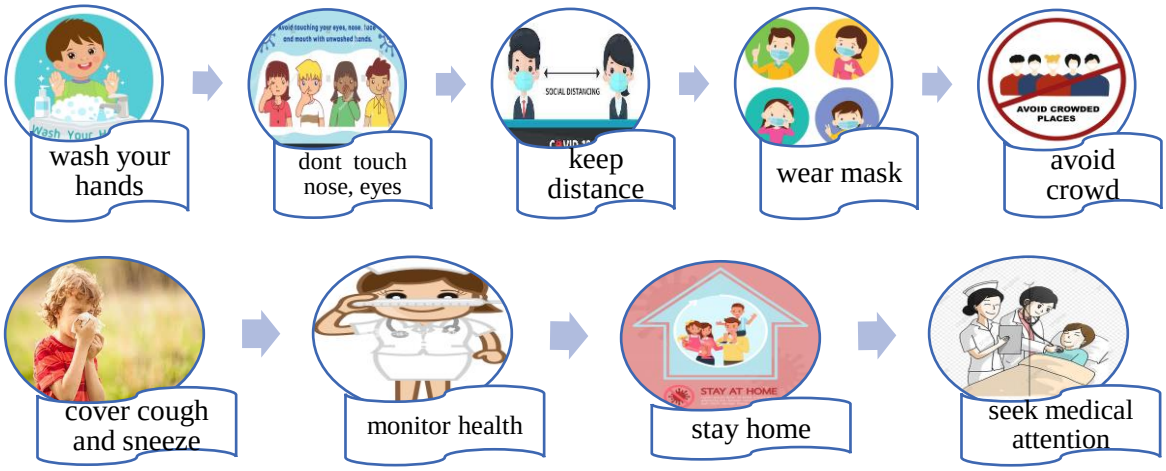

Figure 9. Prevention of COVID-19.

CONCLUSION

COVID-19, also known as SARS-CoV-2, belongs to the Nidovirales order, family Coronaviridae, and subfamily Coronavirinae. The virus has an enveloped and spherical structure with a positive single strand and various accessory, structural, and non-structural proteins. Its life cycle includes attachment, entry, replication, translation, assembly, and release. Some important variants include B.1.1.7, B.1.351, p1, and b.1.617. High cholesterol levels can support COVID-19 entry, while statins with lower cholesterol levels can reduce it. Common symptoms include fever, cough, fatigue, headache, sputum production, diarrhea, and dysphonia. Various tests, such as RT-PCR, RT-LAMP, CT scan, antibodies, and antigen, are used for detection. Vaccines are now available, but they can have side effects like fever, fatigue, and headache. Preventive measures are necessary to prevent its spread.

COMPETING OF INTEREST

The authors declare no competing interests.

REFERENCES

Ahmad, T., M. Khan, T. H. Musa, S. Nasir, J. Hui, D. K. Bonilla-Aldana and A. J. Rodriguez- Morales. 2020. COVID-19: Zoonotic aspects. Travel Med. Infect. Dis. 36, 101607.

Amanat, F. and F. Krammer. 2020. SARS-CoV-2 Vaccines: Status Report. Immunity, 52.

Arsani, P., M. S. Eapen, C. Chia, G. Haug, H. Weber, M., Hassan and S. S. Sohal. 2020. Diagnostic approaches in COVID-19: clinical updates. Expert Rev. Respir. Med.

Arya, R., S. Kumari, B. Pandey, H. Mistry, S. Bihani, A. Das, V. Prashar, G. Gupta, L. Panicker and M. Kumar. 2021. Structural insights into SARS-CoV-2 proteins. Jmb, 433: 166725.

Astuti, I. and Ysrafil. 2020. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): An overview of viral structure and host response. Diabetes & Metabolic Syndrome: Clinical Research & Reviews, 14: 407-412.

Azkur, A. K., M. Akdis, D. Azkur, M. Sokolowska, W. V. D. Veen, M. Bruggen, L. O'Mahony, Y. Gao, K. Nadeau, and C. Akdis. 2020. Immune response to SARS-CoV-2 and mechanism of immunopathological changes in COVID-19. 75(7): 1564-1581.

Chen, S., S. Wang and Y. Chen. 2020. Novel Antiviral Strategies in the Treatment of COVID-19: A Review. Microorganisms, 8(9):1259.

Chen, Y., Q. Liu and D. Guo. 2020. Emerging coronaviruses: Genome structure, replication, and pathogenesis. J. Med. Virol. 92: 418-423.

Dagur, H. S., and S. S. Dhakar. 2020. Genome Organization of Covid-19 and Emerging Severe Acute Respiratory Syndrome Covid-19 Outbreak: A Pandemic. EJMO, 4(2): 107-115.

Diego, J. G. B., P. F. Soto, M. D. Gil, M. B. Garcia, J. L. M. Bellido and A. Muro. 2021. A Simple, Affordable, Rapid, Stabilized, Colorimetric, Versatile RT-LAMP Assay to Detect SARS-CoV-2. Diagnosis, 11: 438.

Ganesh, B., T. Rajakumar, M. Malathi, N., Manikandan, J. Nagaraj, A., Santhakumar, Elangovan, and Y. S. Malik. 2021. Epidemiology and pathobiology of SARS-CoV-2 (COVID-19) in comparison with SARS, MERS: An updated overview of current knowledge and future perspectives. CEGH. 10: 100694.

Garg, H., and P. Khanna. 2021. Covid and cholesterol (C&C): Something to worry about or much ado about anything?

Trends Anaesth. Crit. Care. 36: 39-40.
Guo, C., Y. Bo , C. Lin , B. Li , Y. Zeng, Y., Zhang, M. S., Hossain, J. W. M. Chan, D. W. Yeung, K., Kwok, S. Y. S., Wong, A. K. H., Lau, X. Q. Lao. 2021.Meteorological factors and COVID-19 incidence in 190 countries: An observational study. Sci. Total Environ, 757: 143783.
Guo, Y., Q. Cao, Z. Hong, Y. Tan, S. Chen, H. Jin, K. Tan, D. Wang and Y. Yan. 2020. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak- an update on the status. MMR, 7: 11.
Jin, Y., H. Yang, W. Ji, W. Wu, S. Chen, W. Zhang and G. Duan. 2020. Virology, Epidemiology, Pathogenesis, and Control of COVID-19. Viruses, 12: 272.
Joshi, S., J., Parker, A. Ansari, A. Vora, D. Talwar, M. Tiwaskar, S. Patil and H. Barkate. 2021. Role of Favipiravir in the treatment of COVID-19. IJID, 102: 501-508.
Kadam, S. B., G. S. Sukhramani, P. Bishnoi, A. A. Pabole, and V. T. Barkar. 2021. SARS-CoV-2, the pandemic coronavirus: Molecular and structural insights. J. Basic Microbiol. 61: 180-202.
Keni, R., A. Alexander, P. G. Nayak, J. Mudgal and K. Nandakumar. 2020. COVID-19: Emergence, Spread, Possible Treatments, and Global Burden. Frontiers in Public Health, 8:216.
Kevadiya, B. D., J. Machhi, J. Herskovitz, M. D. Oleynikov, W. R. Blomberg, N. Bajwa, D. Soni, Das, S., Hasan, M., Patel, M., Senan, A. M., Gorantia, S., McMiliian, J., Edagwa, B., Eisenberg, R., Gurumurthy, C. B., Reid, S. P. M., C. Punyadeera, L. Chang and H.E. Gendelman. 2021. Diagnostics for SARS-CoV-2 infections. Nat. Mater. 20: 593-605.
Khan, A. A., F. S. Lodhi, U. Rabbani, Z. Ahmed, S. Abrar, S. Arshad, S. Irum and M. I. Khan. 2021. Impact of Coronavirus Disease (COVID-19) Pandemic on Psychological Well-Being of the Pakistani General Population. FPSYT. 11, 564364.
Kopel, J., H. Goyal and A. Perisetti. 2021. Antibody tests for COVID-19. Baylor University Medical Center Proceedings, 34(1): 63-72.
Kumar, S., R. Nyodu, V. Maurya and S. Saxena 2020. Morphology, Genome Organization, Replication, and Pathogenesis of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS CoV-2). Coronavirus Disease 2019, 23-31.
Lebeau, G., D. Vanger, E. Frumence, F. Ah-Pine, X. Guillot, E. Nobecourt, L. Raffray and P. Gasque. 2020. Deciphering SARS-CoV-2 Virologic and Immunologic Features. Int. J. Mol. Sci, 21(16): 5932.
Lin, Y., Q. Duan, Y., Zhou, T. Yuan, P. Li, T. Fitzpatrick, L. Fu, A. Feng, G. Luo, Y. Zhan, B. Liang, S. Fan, S. Lu, B. Wang, Z. Wang, H. Zhao, Y. Gao, M. Li, D. Chen, X. Chen, Y. Ao, L. Li, W. Cai, X. Du, Y. Shu and D. Zou. 2020. Spread and Impact of COVID-19 in China: A Systematic Review and Synthesis of Predictions From Transmission-Dynamic Models. Front. Med. 7:321.
Liu, Y., R. Kuo and S. Shih. 2020. Covid-19: The first documented coronavirus pandemic in history. Biomedical Journal, 43(4): 328-333.
Mahdy, M., A., A., W. Younis and Z. Ewaida. 2020. An Overview of SARS-CoV-2 and Animal Infection. Front. vet. sci. 7.
Malik, J. K., A. Kumar and H. S. Professor. 2020. Epidemiology of Novel Corona Virus (Covid-19): A Review. Journal of Clinical/ Pharmaco- J. Epidemiol. 2(2): 3742906.
Miao, H., H. Li, Yao, M. Wu, C. Lu, J. Wang, M. Tian, Y. Li, P. Luo, J. GU, B. Yuan, S. Wang, X. Zhao, W. Gan and D. Zhao. 2020. Update on recommendations for the diagnosis and treatment of SARS-CoV-2 infection in children. Eur. J. Clin. Microbiol. Infect. Dis. 39: 2211-2223.
Mousavizadeh, L., and S. Ghasemi. 2021. Genotype and phenotype of COVID-19: Their roles in pathogenesis. J. Microbiol. Immunology and Infection, 54: 159-163.
Murgolo, N., A. G. Therien, B. Howell, D. Klein, K. Koeplinger, L. A. Liberman, G. C., Adam, J. Flynn, P. McKenna, G. Swaminathan, D. G. Hazuda and D. B. Olsen. 2021. SARA-CoV-2 Tropism, entry, replication, and propagation: Consideration for drug discovery and development. Plos Pathog. 17(2): 1009225.
Naqvi, A. A. T., K. Fatima. T. Mohammad, U. Fatima, I. K. Singh, A. Singh, S. M. Atif, M. Hairprased, G. U. Hasan, and M. I. Hassan. 2020. Insights into SARS-CoV-2 genome, structure, evolution, pathogenesis and therapsis: Structural genomics approach. Elsevier public health emergency collection, 1866(10): 165878.Parasher, A. 2020. Covid-19: Current understanding of its pathophysiology, clinical presentation and treatment. 1-9.
Pillaiyar, T. 2015. Middle East Respiratory Syndrome-Coronavirus (MERS-CoV): An Updated Overview and Pharmacotherapeutics. Medicianl Chemistry, 5: 361-372.Rasheed, M. A., Raza, S., & Rashid, M. K. 2020. Transmission Potential and Severity of COVID-19 in Pakistan.
Prince, T., S. L. Smith, A. D. Radford, T. Solomon, G. L. Hughes and E. Patterson. 2021. SARS-CoV-2 Infections in Animals: Reservoirs for Reverse Zoonosis and Models for Study. Viruses, 13: 494.
Radenkovic, D., S. Chawla, M. Pirro, A. Sahebkar and M. Banach. 2020. Cholesterol in Relation to COVID-19: Should We Care about it? Journal of Clinical Medicine, 2: 1909.Sahin, A. R., Erdogan, A., Mutlu, A. P., Dineri, Y., Cakirci, A.Y., Senel, M.E., Okyay, A. R., &Tasdogan, A. M. 2020. 2019 Novel Coronavirus (COVID-19) Outbreak: A Review of the Current Literature. EJMO, 4(1): 1-7.
Rahman, H. S., M. S. Aziz, R. H. Hussein, H. H. Othman, S. H. S. Omer, E. S. Khalid, N. A. Abdulrahman, K. Amin

and R. Abdullah. 2020. The transmission modes and sources of COVID-19: A systematic review. Int. Surg. J. 26: 125-136.
Rothan, H. A., and S. N. Byrareddyy. 2020. The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. J. Autoimmun. 109: 102433.
Sattar, Y., W. Ullah, H. Rauf, H. H. Virk, S. Yadav, M. Chowdhury, M. Connerney, S. Mamtani, M. Pahuja, R. Patel, T. Mir, T. Almas, H. M. Pacha. and M. C. Alraies. 2020. COVID-19 cardiovascular epidemiology, cellular pathogenesis, clinical manifestations and management. IJC Heart & Vasculature. 29: 100589.
Shah, V., R. Keniya, A. Shridharani, M. Punjabi, J. Shah and N. Mehendale. 2021. Diagnosis of COVID-19 using CT scan images and deep learning techniques. Emerg. Radiol. 28: 497-505.
Sofi, M. S., A. Hamid and S. U. Bhat. 2020. SARS-CoV-2: A critical review of its history, pathogenesis, transmission, diagnosis and treatment. Biosaf. Health, 2(4): 217-225.
Tannak, N. A., L. Novotny and A. Alhunayan. 2020. Remdesivir-Bringing Hope for COVID-19 Treatment. Sci. Pharm. 88: 29.
Tanu S., 2020. A Review of Coronavirus Disease-2019 (COVID-19). The Indian Journal of Pediatric, 87(4): 281–286
Tiwari, R., K. Dhama, K. Sharun, M. I. Yatoo, Y. S. Malik, R. Singh, I. Michalak, R. Sah, D. K. B. Aldana and A. J. R. Morales. 2020. COVID-19: animals, Veterinary and zoonotic links. Vet. Ext. Q. 40(1): 169-182.
To, K.K., I. F. Hung, J. F. Chan and K. Y. Yuen. 2013. From SARS coronavirus to novel animal and human coronaviruses. J. Thorac. Dis. 2072-1439.
Tu, Y., C. Chien, A. Yarmishyn, Y. Lin, Y. Luo, Y. Lin, W. Lai, D. Yang, S. Chou, Y. Yang and M. Wang. 2020. A Review of SARS-CoV-2 and the Ongoing Clinical Trials. International Journal of Molecular Science, 21: 2657.
Walensky, R. P., H. T. Walke and A. S. Fauci. 2021. SARS-CoV-2 Variants of concern in the United States- Challenges and Opportunities. JAMA. 325(11): 1037-1038.
Wang, H., X. Li, T. Li. S. Zhang, J. Wang, X. Wu and J, Liu. 2020. The genetic sequence, origin, and diagnosis of SARS-CoV-2. Eur. J. Clin. Microbiol. Infect. Dis. 39: 1629-1635.
Wiwanitkit, V. 2020. Atypical modes of COVID-19 transmission: how likely are they? Epidemiology and Health, 42.
Yadav, R., J. K. Chaudhary, N. Jain, P. K. Chaudhary, S. Khanra, P. Dhamija, A. Sharma, A., Kumar and S. Handu. 2021. Role of Structural and Non-Structural Proteins and Therapeutic Targets of SARS-CoV-2 for COVID-19. Cells, 10:821.
Yuan, F., S. Jiang and Z. Li. 2020. Analysis of Possible Intermediate Hosts of the New Coronavirus SARS-CoV-2. Front. vet. sci. 7: 379.
Zhai, P., Y. Ding, X. Wu, J. Long, Y. Zhong and Y. Li. 2020. The epidemiology, diagnosis and treatment of COVID-19. Int. J. Antimicrob. Agents. 55: 10955.
Zhang, Z., Y. Hou, D. Li and F. Li. 2021 Diagnostic efficiency of anti-SARS-CoV-2 igG/igM test for COVID-19: A meta-analysis. J. Med. Virol. 93(1): 366-374.
Zhao, J., W. Cui and B. Tian. 2020. The potential intermediate Hosts for SARS-CoV-2. Front. Microbiol, 11.