

Updates on COVID-19: Virology, Etiology, Epidemiology, Pathogenesis, Diagnosis, Transmission and Prevention

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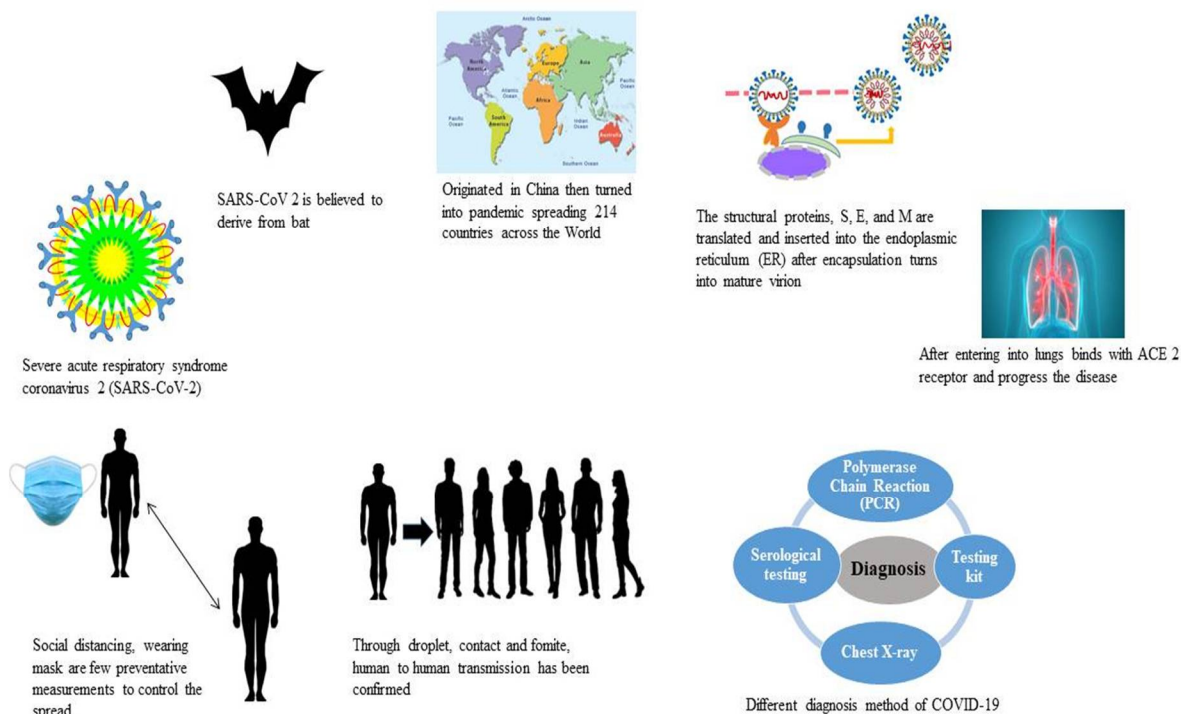
Abstract

COVID-19 is a pandemic malady caused by SARS-CoV-2, a novel coronavirus. It is a global threat that has affected 223 countries and territories all over the world. According to the WHO report as of April 06, 2022 coronavirus has affected 492,189,439 people globally with 6,159,474 confirmed deaths. The pandemic of COVID-19 has badly affected Bangladesh likewise many other countries of the world. According to Worldometer report on 06 April 2022 the number of confirmed cases of COVID-19 were 1,951,903 with 29,123 deaths, and 1,886,036 COVID-19 recoveries in Bangladesh. After originating from China, this notorious virus has been spread to almost all the countries of the world. Its spike protein aids in binding with the ACE2 receptors of the cell membrane resulting in cell entry, replication, and induction of inflammatory and pro-inflammatory responses leading to the pathogenic condition. The novel COVID-19 virus has structural and genetic similarity with its predecessors specially SARS-CoV and MERS-CoV. This review presents the existing literature on COVID-19 and discusses different aspects of COVID-19 including virology, etiology, epidemiology, pathogenesis, diagnosis, transmission and susceptibility and preventive measures of COVID-19.

Key words: COVID-19, coronavirus disease, virology, etiology, epidemiology, pathogenesis, diagnosis, transmission, prevention, infectious disease, COVID-19 pandemic.

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Graphical abstract:**Introduction**

The brooding period of SARS-CoV-2 (COVID-19) infection ranges from 1 to 14 days, mostly from 3 to 7 days (Shen *et al.*, 2020). This period relies on the patient's age and immune system, mostly aged men are at more risk than women (Wang *et al.*, 2020a; Jin *et al.*, 2020; Onder *et al.*, 2020). Initially, infected COVID-19 patients show some onset of symptoms like fever, cough, and myalgia/fatigue. Sputum production, hemoptysis, headache, dysentery, dyspnea and lymphopenia are less occurring symptoms and in severe cases, RNAemia, acute respiratory distress syndrome, cardiac injuries, and incidence of ground-glass opacities have been found that gradually led to death (Huang *et al.*, 2020).

As of 06 April 2022, coronavirus has spread in 223 countries and territories with 492,189,439 confirmed cases of COVID-19 infections and 6,159,474 deaths (WHO, 2022). The COVID-19 pandemic has also affected Bangladesh likewise

other countries. On 8 March 2020, the first case of COVID-19 was confirmed in Bangladesh. According to the Directorate General of Health Services (DGHS), there were 20,065 COVID-19 confirmed cases by rt-PCR test between 8 March and 15 May 2020, including 298 related death cases where the case fatality rate (CFR) was 1.48%. Here, the highest confirmed case was identified on 15 May which was 1202 and highest death was noted on 13 May 2020 (Hossain *et al.*, 2020). According to Worldometer report on 06 April 2022, there have been 1,951,903 confirmed cases of COVID-19 with 29,123 deaths and 1,886,036 COVID-19 recoveries in Bangladesh (Coronavirus cases: Bangladesh, 2022).

To prevent the infection as well as to be aware of its treatment, transmission, and other aspects, detailed information on COVID-19 is important. That's why, we have aimed to produce this review which presents the existing literature on COVID-19 discussing different aspects of COVID-19 including virology, etiology, epidemiology, pathogenesis, diagnosis,

transmission, susceptibility and preventive measures of COVID-19.

Virology and replication of SARS-CoV-2: After the crown-like morphological observation under the electronic microscope, this virus was termed 'coronavirus' in 1968 (Virology: Coronaviruses, 1968)

Taxonomy of coronavirus (Gorbalenya *et al.*, 2020; Korsman *et al.*, 2012)

Realm:	<i>Riboviria</i>
Order:	<i>Nidovirales</i>
Sub-order:	<i>Cornidovirineae</i>
Family:	<i>Coronaviridae</i>
Sub-family:	<i>Orthocoronavirinae</i>
Genus:	<i>Beta corona virus</i>
Sub-genus:	<i>Sarbecovirus</i>
Species:	Severe acute respiratory syndrome coronavirus (SARS)

Genome and virion: Coronavirus (CoV) genomes are consisted of non-segmented positive polar RNA strands made of 27 and 32,000 kilobases nucleotide approximately making it the biggest incessant RNA genome amid mammalian viruses (Woo *et al.*, 2009; Li *et al.*, 2020). The virion RNA is fused with the basic nucleocapsid (N) protein with a nucleocapsid (N) protein situated inside the

phospholipid bilayers and two different types of spike proteins (S) made with peplomers on the surface of the virus gives it crown like shape, hydrophobic envelope protein (E) and 16 nonstructural proteins (nsp) (Weiss *et al.*, 2005; Weiss *et al.*, 2011; de Wilde *et al.*, 2018; Li *et al.*, 2020).

Genotypically and serologically the CoVs are classified into 4 subfamilies which are α , β , γ , and δ -coronavirus. α - and β -CoVs are responsible for human infections (Weiss *et al.*, 2011; Li *et al.*, 2020). In case of alpha and beta-coronaviruses an identical reserved sequence UUUAAAC has been found at the genome positions 12-14000. A papain-like protease (Plpro), a helicase, two methyltransferases, a RNA-dependent RNA polymerase (RdRp), main protease (Mpro), and quite a few innate immunity antagonists (Perlman and Netland, 2009) are transcribed through ribosome (Brian and Baric, 2005).

On 30th December 2019 the SARS-CoV-2 strain was secluded for the first time in bronchoalveolar lavage fluid (BALF) (Zhou *et al.*, 2020) from three COVID-19 patients admitted to Wuhan Jinyintan Hospital (Zhu *et al.*, 2020a). After sequence analysis and prompt research, SARS-CoV-2 has been found as a member of β -CoVs (Zhou *et al.*, 2020; Li *et al.*, 2020).

The classification of coronaviruses has been listed in table 1.

Table 1. Classification and types of coronavirus.

Genera	Types	References
Alphacoronavirus	Human corona virus: HCoV-NL63 and HCoV-229E, Feline corona virus (FCoV), canine coronavirus (CCoV)	Ashour <i>et al.</i> , 2020; Woo <i>et al.</i> , 2009
Betacoronavirus	HCoV-OC43, HCoVHKU1, Severe acute respiratory syndrome coronavirus (SARS-CoV), MiddleEast respiratory syndrome coronavirus (MERS-CoV), BtCoV-HKU4, BtCoV-HKU5, BtCoV-HKU9	Ashour <i>et al.</i> , 2020; Woo <i>et al.</i> , 2009
Gammacoronavirus	Avian coronavirus, IBV	Ashour <i>et al.</i> , 2020; Woo <i>et al.</i> , 2009
Deltacoronavirus	Night heron CoV, BuCoV-HKU11	Ashour <i>et al.</i> , 2020; Woo <i>et al.</i> , 2009
Omicron	Mutated variant of SARS-CoV-2 designated B.1.1.529	Cascella <i>et al.</i> , 2022

As per the epidemiological update of the World Health Organization in December 2021, five SARS-

CoV-2 coronavirus variants have been identified since the start of the pandemic (Cascella *et al.*, 2022).

- **Alpha:** This variant is designated as B.1.1.7. In late December 2020, this is the first variant described in the United Kingdom (UK).
- **Beta:** This variant is designated as B.1.351 which was first reported in South Africa in December 2020.
- **Gamma:** This variant is designated as P.1 which was originally discovered in Brazil in early January 2021.
- **Delta:** This variant is designated as B.1.617.2 which was first found in India in December 2020.
- **Omicron:** This variant is designated as B.1.1.529 which was discovered in November 2021 in South Africa.

SARS coronavirus (SARS-CoV) and MERS coronavirus (MERS-CoV) belongs to β -CoVs group (Weiss and Leibowitz, 2011). According to the genome-phylogenetic analysis, SARS-CoV-2 shares 79.5%/79.6% (Zhou *et al.*, 2020) and 50% sequence identity to SARS-CoV and MERS-CoV, respectively (Zhu *et al.*, 2020a; Zhou *et al.*, 2020; Lu *et al.*, 2020). Though, 94.6% similarities in sequence have been found between the seven conserved replicas domains in ORF1ab of SARS-CoV-2 and SARS-CoV (Zhou *et al.*, 2020) and 96% identical with a bat coronavirus (Zhou *et al.*, 2020). SARS-CoV-2 shares less than 90% genome sequence with other β -CoVs (Zhu *et al.*, 2020) which concludes that SARS-CoV-2 belongs to the lineage B (Sarbecovirus) of β -CoVs (Wu *et al.*, 2020). A representative diagram of SARS-CoV-2 has been presented in figure 1.

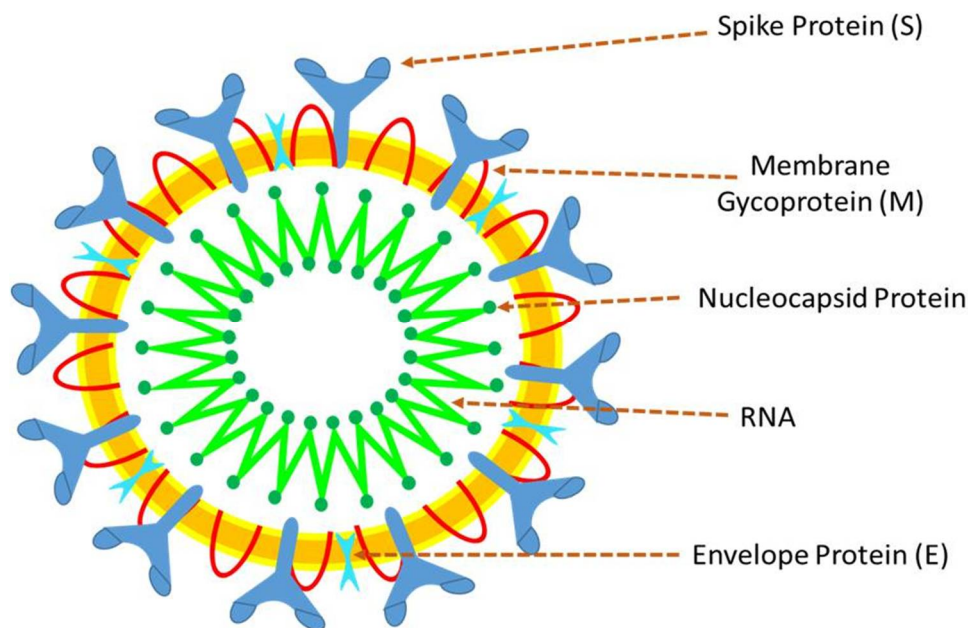


Figure 1. A representative diagram of SARS-CoV-2.

Physicochemical properties: Coronaviruses are minute in size with a diameter of 65~125 nm (Shereen *et al.*, 2020) and round or oval. It can be inactivated if heated at 56 °C for 30 min or put under UV rays. The SARS-CoV-2 virus is mostly sensitive to 75% ethanol, diethyl ether, chloroform, chlorine, and peracetic acid. According to scientific reports,

SARS-CoV-2 is less stable on copper and more stable on plastic and stainless steel. The virus was found on cardboard for up to 72 hours (van Doremalen *et al.*, 2020).

Replication and transcription: The RNA synthesis of the virus replicates the translation and assembly of the viral replicase complexes (Malik,

2020) and produces genomic and sub-genomic RNAs as by-products (Malik, 2020). Among these two, the Sub-genomic RNAs play the role of mRNAs for the structural and accessory genes (Malik, 2020). It resides downstream of the replicase polyproteins (Malik, 2020). Via negative-strand intermediates, both the byproducts i.e. genomic and sub-genomic RNAs are produced (Malik, 2020). About 1% of these intermediates are abundant as positive sense counter parts (Malik, 2020). In their structure, they

both have poly-uridylyate and anti-leader sequences (Malik, 2020). Recombination depend on the strand switching ability of the RNA-dependent RNA polymerase (RdRp) and supposed to be a significant part of the viral evolution (Malik, 2020). It is the target base for RNA recombination which is a reverse genetics weapon used to engineer viral recombinants at the 3' end of the genome (Malik, 2020). The replication of SARS-CoV-2 has been presented in figure 2.

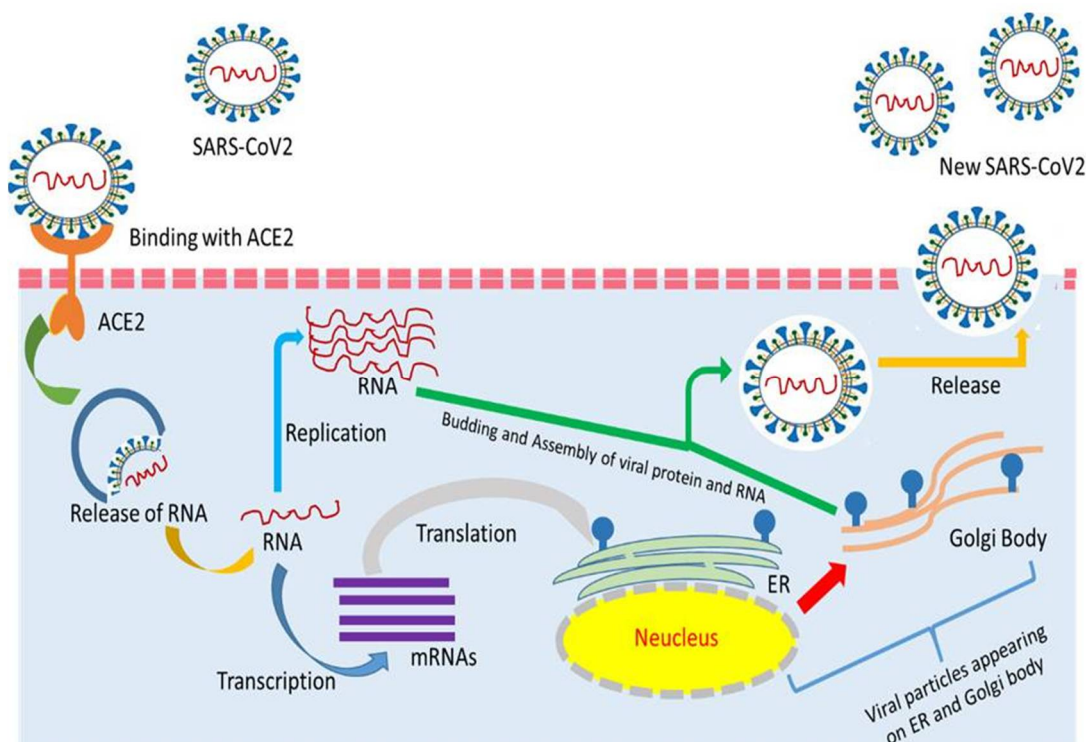


Figure 2. Replication of SARS-CoV-2.

Assembly and release: After replication and subgenomic RNA synthesis, the three proteins S, E, and M are translated and introduced into the endoplasmic reticulum (ER). The encapsulated viral genome forms a mature virus after budding (De Haan *et al.*, 2003). The protein M then guides protein-protein interaction for assembly (De Haan *et al.*, 2003). After the assembly, virions go to the cell surface in vesicles and follow exocytosis (Malik, 2020). Depending on the ability to mutate, recombine, infect and produce new strains,

coronavirus keeps evolving, emerging, and causing new pandemics (Malik, 2020).

Etiology: The initial report reveals that SARS-CoV-2 virus shares 88% sequence characteristics with SARS-like coronaviruses derived from two bats, it is more different than SARS-CoV (Lu *et al.*, 2020; Chan *et al.*, 2020). That is why this virus was named 2019-novel coronavirus (2019-nCoV) (Lu *et al.*, 2020; Chan *et al.*, 2020). One proof to support the statement that the origin of this virus can be bat is the homology of the ACE2 receptor from a range of

animal species (Wan *et al.*, 2020). It was mentioned in another article that the viruses contribute to an intact open reading frame on gene 8 and that another sign of bat-origin CoVs is that the amino acid sequence of the potential receptor-binding domain is similar to that of SARS-CoV, suggesting that these viruses may use the same receptor (Ren *et al.*, 2020).

This virus is an envelope virus and possesses single-stranded ribonucleic acid and is named after its crown-like figure in the outer layer formed due to 9-12 nm long surface spike (Zu *et al.*, 2020). Some articles have stated that SARS-CoV 2 has four major structural protein which is encoded by the viral genome on the envelope (Kirchdoerfer *et al.*, 2016; Xu *et al.*, 2020). In addition, another one is the spike protein and this protein binds to the angiotensin-converting enzyme 2 (ACE2) receptor (Kirchdoerfer *et al.*, 2016; Xu *et al.*, 2020). WHO divided the COVID-19 virus as a β CoV of group 2B (Hui *et al.*, 2020). According to this article, this virus demonstrated 99.98% sequence identity (Hui *et al.*, 2020). In another article, it showed 99.8-99.9% nucleotide identity (Lu *et al.*, 2020), and also, it showed 50% of identity with the MERS virus (Lu *et al.*, 2020). According to phylogenetic analysis, bats can be actual hosts of this virus (Chan *et al.*, 2020). Other animals sold at the seafood market in Wuhan could have played the role of an intermediate hosts (Chan *et al.*, 2020). This intermediary animal might have expedited the emergence of this virus in humans (Chan *et al.*, 2020). As the possible intermediary animals, the name of pangolin (Lam *et al.*, 2020) or other wild animals (Zhang *et al.*, 2020) came forward. This virus is the seventh member of the coronavirus family and customs clade within the subgenus sarbecovirus (Zhu *et al.*, 2020a). As per Zhu *et al.* (2020b), the new coronavirus may have had bats and minks as its two potential hosts, with the latter serving as the virus's intermediary host (Cheng *et al.*, 2020).

A complete viral genomic analysis conducted by Roujian Lu and Co-workers (Lu *et al.*, 2020; Chan *et al.*, 2020) disclosed that SARS- coronavirus 2 shows 88% similar sequence identical to bat derived severe

acute respiratory syndrome (SARS)-like coronaviruses such as bat-SL-CoVZC45 and bat-SL-CoVZXC21. In another cutting edge paper (Chan *et al.*, 2020), it is found that the identical genome sequence of 2019-nCoV and bat SARS-related-CoV SLCoVZXC21 is approximately 89%. However, SARS-CoV-2 diligently differs from SARS-CoV and MERS-CoV respectively in percentages of 79 and 50 (Lu *et al.*, 2020; Chan *et al.*, 2020). The phylogenetic analysis exposed that 2019-nCoV is a novel lineage B Beta coronavirus which falls under subgenus Sarbecovirus. Also, this virus is a typical RNA virus and possesses an average evolutionary rate of roughly (Lu *et al.*, 2020; Chan *et al.*, 2020). This study clearly demonstrated that more than 99.9% of the sequence was identical to different patients. Thus, it also lends support to the fact that this virus has originated from one source within a miniscule amount of time and comparatively promptly (Lu *et al.*, 2020; Chan *et al.*, 2020).

Epidemiology: The expression of COVID-19 disease came into light on December, 2019 (Cui *et al.*, 2019). At the beginning, very low number of morbidity existed. However, then it started increasing and reaching to peak in the mid of January 2020 (Cui *et al.*, 2019). Then eventually with time it showed remarkable increase of infected people and death rate (Cui *et al.*, 2019). Numbers started getting increased outside of Hubei province of China on the eve of lunar Chinese New Year and drew attention around the world (Cui *et al.*, 2019). COVID-19 flare-up worldwide has come perturbing to everyone, for its increased rate of spread as well as for the high case of fatality rate reported so far. This uncertain, unknown and unpredicted disease could bell the global alarm with more force than it was ever expected before (Petropoulos *et al.*, 2020). The intensity of COVID-19 infection changes in dimension can be explained with the three opinions from the experts: Elon Musk said that "The coronavirus panic is dumb" (Bariso, 2020).

The mathematician Nassim Nicholas Taleb told, "Saying the coronaviruspanic as dumb is what's dumb" (The Economic Times Panache. 2020), and

Bill Gates said “I hope it’s not that bad, but we should assume it will be until we know otherwise”

(Bariso, 2020). The epidemiological differences of coronaviruses are presented in table 2.

Table 2. Epidemiological differences between SARS CoV 2, SARS-CoV and MERS CoV (Hossain et al., 2020).

Characteristics	SARS CoV 2	SARS CoV	MERS CoV
Reproduction number	2.68	2-5	>1
Incubation	6.4 days (0-24 days)	4.6 days	5.2 days
Transmission	Transmitted through physical contact among human, aerosol droplets, contact, fomites, nosocomial transmission, zoonotic transmission.	Transmission occurs via person to person contact, aerosol droplets, opportunistic airborne, nosocomial, faecal-oral and zoonotic transmission	The virus is being transmitted through respiratory, aerosol, nosocomial transmission, zoonotic transmission, human-to human transmission,
Hosts	Bats as natural hosts, pangolin as intermediate and humans as terminal host.	Chinese horseshoe bats as natural hosts, masked palm civets as intermediate and humans as terminal host.	Bats are natural hosts, dromedary camels are intermediate hosts and humans are terminal hosts.

SARS-CoV-2= Severe acute respiratory syndrome coronavirus 2; SARS-CoV= Severe acute respiratory syndrome coronavirus; MERS-CoV= Middle East respiratory syndrome coronavirus.

Pathogenesis and susceptibility: Proteins of coronavirus play a very important role in pathogenesis. The spike (S) protein of coronavirus is a type I glycoprotein (Sturman et al., 1977). The cysteine-rich protein with cytoplasmic tail is required for fusion (Chang et al., 2000). The SARS-CoV spike protein induces interleukin-8 (IL-8) by activating MAPK and AP-1 in the lungs, which leads to pathogenesis. (Chang et al., 2004). Hemagglutinin-Esterase (HE) Protein creates a second type of spike protein smaller than spike protein peplomers situated on the envelope of coronavirus (Kienzle et al., 1990; Wesley et al., 1991). Though HE is not essential for cell replication its spike is required to mediate host cell entry. The membrane (M) protein is the most prolific virion membrane protein. Glycosylation is not requisite for viral assembly or infection (De Haan et al., 2003), but the state of glycosylation of M protein assists along with S and HE proteins in the interaction of the virus with the host cell (Laude et al., 1992). nucleocapsid (N) Protein is the structural protein that acts in pathogenesis along with transcription (Yount et al., 2002; Yount et al., 2003) and it was also found to increase the replication of HCoV-229E genome RNA (Schelle et al., 2005).

Another essential membrane protein of coronavirus is small envelope (E) protein. It plays a key role in viral assembly and together with M forms viral particles that take part during infection and induce cell apoptosis (Yu et al., 1994).

Hypothetical pathogenesis: Details of the cellular responses of this virus are not known; hence we can only postulate an outline of the series of changes it brings when it enters the host body based on SARS-CoV (Mason, 2020) as SARS-CoV-2 has 79.5% similarities with the genomic sequence of the SARS-CoV that had caused pandemic during 2002-2003 (Rabi et al., 2020).

In the first step, receptor-mediated virus entry has occurred into the host cell. Angiotensin-converting enzyme-2 (ACE2) protein was found to be the SARS-CoV and SARS-CoV-2 receptor. (Li et al., 2003; Kuba et al., 2005; Xiao et al., 2020) and expressed in copious on the glandular cells of the heart, kidneys, gastric, duodenal and rectal tissue supports the entry of SARS-CoV-2 into the host cells (Xiao et al., 2020).

The type 2 transmembrane protease TMPRSS2 mediates the degradation of ACE-2 and activation of the spike protein once the CoV-2 spike

protein (S) attaches to the ACE-2 receptor (Glowacka *et al.*, 2011; Heurich *et al.*, 2014). Thus, it promotes the fusion of the viral membranes (Hofmann *et al.*, 2004). Post viral entry, virus-specific RNA, N protein, envelope glycoproteins, M protein, S-protein, and 8 other proteins of unspecified function are synthesized in the cytoplasm replicating new virions (Weiss and Navas-Martin, 2005; Hofmann *et al.*, 2004).

First one or two days few patients may remain asymptomatic (Mason, 2020) and doesn't show any symptoms, and some patients show non-respiratory symptoms such as acute liver and heart injury, kidney failure and diarrhea (Huang *et al.*, 2020; Cheng *et al.*, 2020; Guan *et al.*, 2020a; Guan *et al.*, 2020b; Wang *et al.*, 2020b). Due to the high concentration of ACE2 in the nasal mucosa, bronchi, lung, heart, esophagus, kidney, stomach, bladder and ileum these human organs are most vulnerable to SARS-CoV-2 infections (Zou *et al.*, 2020).

ARDS (Acute respiratory distress syndrome) is a fatal condition. ACE2, interleukin 10 (IL-10), tumor necrosis factor (TNF), vascular endothelial growth

factor (VEGF) and amplified levels of plasma IL-6 & IL-8 are associated with the development of ARDS (Meyer *et al.*, 2013). In this state, oxygen cannot get into the lungs and blood circulation making it the most common reason of death among patients with respiratory diseases and acute lung injury (Thompson *et al.*, 2017).

Pulmonary pathological changes significantly resemble SARS (Ding *et al.*, 2003) and MERS (Ng *et al.*, 2016). In fatal reports with COVID-19, unlike SARS and MERS, it was found a large amount of mucus secretion in both lungs. Some inflammatory responses by SARS-CoV-2 may cause fatality in patients such as rapid viral replication and cellular damage, virus-induced ACE2 down-regulation shedding and antibody dependent enhancement (ADE) (Fu *et al.*, 2020). Acute lung injury can result from the renin-angiotensin system's (RAS) dysfunction, which can be brought on by the downward regulation and shedding of ACE2 (Fu *et al.*, 2020). The immune response against SARS-CoV-2 has been presented in figure 3.

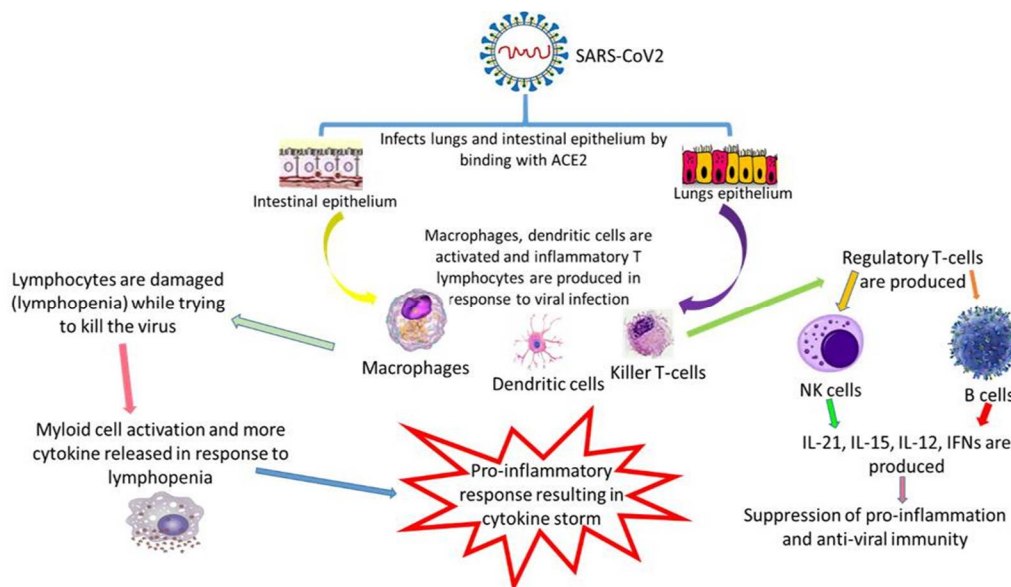


Figure 4. Immune response against SARS-CoV-2.

In the blood plasma of SARS-CoV-2 infected patients, significant amount of cytokines and chemokines were found that includes IL1- β , IL1RA,

IL7, IL8, IL9, IL10, FGF2, GCSF, GMCSF, IFN γ , IP10, MCP1, MIP1 α , MIP1 β , PDGFB, TNF α , IL2, IL7, IL10, GCSF, IP10, MCP1, MIP1 α and TNF α .

(Huang *et al.*, 2020a; Huang *et al.*, 2020a). By altering the inflammatory responses, the interaction of FcR with anti-S protein-neutralizing antibodies (anti-S-IgG) can result in severe lung injury (Liu *et al.*, 2019), also sudden death is mostly seen in patients who neutralize antibodies suffer from consistent inflammation and ARDS (Fu *et al.*, 2020).

Over activation of T cells, rich concentrations of proinflammatory CD4 T cells and cytotoxic granules

CD8 T cells (Xu *et al.*, 2020a; Xu *et al.*, 2020b) and lymphopenia are some common features of COVID-19 (Huang *et al.*, 2020a; Huang *et al.*, 2020b) causing the severity of disease and mortality in patients. Recently, SARS-CoV-2 has also been found pathogenic to testicular tissues implying fertility risk in young patients (Fan *et al.*, 2021). The postulated pathogenesis of SARS-CoV-2 infection is graphed in Figure 4.

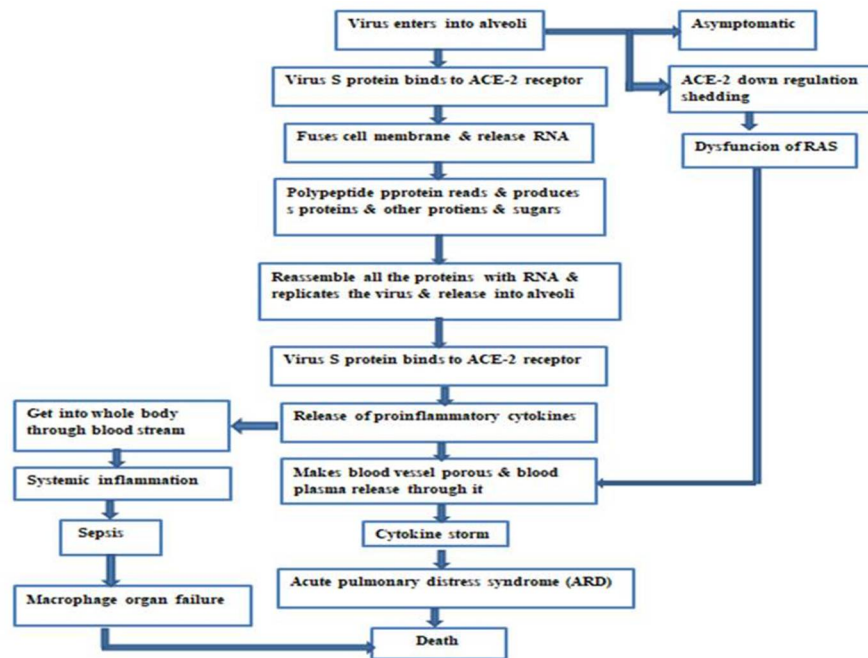


Figure 5. An overview of SARS-CoV-2 pathogenesis.

Susceptibility: In general, coronaviruses in maximum cases affect vertebrates which include human birds, bats, snakes, mice and other wild animal (Balasuriya *et al.*, 2013). From 1960 to date, seven coronaviruses have been identified which infect humans and among these, 229E and OC43 are found to show low virulence infection in 15 to 29% (Su *et al.*, 2016; Isaacs *et al.*, 1983). In the earlier time, HCoV-NL63, α -CoVs HCoV-229E, HCoV-OC43, and β -CoVs HCoV-HKU1 have been reported to show mild symptoms in humans (Graham & Baric, 2010). However, some β -CoVs have successfully gone across the species barrier by causing respiratory illness resulting in life-threatening pneumonia transmitting to human from animal (Stawiski *et al.*,

2020). Such types of HCoVs severe acute respiratory syndrome corona virus or SARS-CoV (2002 to 2003) (Holmes, 2003), Middle East respiratory syndrome corona virus or MERS-CoV (2012) (Li, 2016), and most recently, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which has taken the shape of global pandemic (2019 till exists) (Chan *et al.*, 2020). In a very recent article, the susceptibility of SARS CoV 2 in some animals has been described (Shi *et al.*, 2020). It is stated that SARS CoV 2 replicates poorly in dogs, pigs, chickens and ducks (Shi *et al.*, 2020). However, this statement showed less significance in case of ferrets and cats (Shi *et al.*, 2020). The article also claimed that experimentally

cats are generally susceptible to airborne infection (Shi *et al.*, 2020).

With time, in contrary to SARS-CoV's spike glycoprotein (S-protein), the S protein of SARS CoV 2 which binds to angiotensin converting enzyme 2 (ACE2) has achieved mutation and thus has taken a shape of highly contagious disease (Stawiski *et al.*, 2020). In a recent study, the possible alteration in ACE2 and S-protein interaction has been brought by doing ACE2 polymorphisms and thus an assessment has been conducted to test host susceptibility to SARS-CoV2 (Stawiski *et al.*, 2020). In that report the S-protein interacting synthetic mutant map of ACE2 has been used to identify natural ACE2 variants which can alter the virus-host interaction (Stawiski *et al.*, 2020). Some variants which can increase susceptibility are I21V, K26R, E23K, S19P, N64K, T27A, Q102P, T92I and H378R whereas; N33I, E37K, K31R, D38V, H34R, E35K, Y50F, N51S, M62V, K68E, F72V, Y83H, G326E, D509Y, G352V, Q388L and D355N are the putative ACE2 variants which can decrease the susceptibility (Stawiski *et al.*, 2020). Another in silico study claims that ACE2 single-nucleotide polymorphisms (SNPs) have an impact on SARS-CoV-2 spike glycoprotein (Calcagnile *et al.*, 2020). They have found that S19P and K26R which were mostly diffused SNPs worldwide are common in African and European people respectively (Stawiski *et al.*, 2020).

It is also claimed that the use of ACEi and ARBs can enhance the high expression of ACE2 leading to the accelerated entry of SARS-CoV-2 and so, they recommended cessation of such drugs (Fang *et al.*, 2020a; Fang *et al.*, 2020b; Kuster *et al.*, 2020; Yilmaz *et al.*, 2020). They also claimed that the people who take thiazolidinediones and ibuprofen are over-expressive to this disease as it increases ease expression of ACE2 (Fang *et al.*, 2020a; Kuster *et al.*, 2020, Yilmaz *et al.*, 2020). However, these claims have been denied by many scientists as no strong evidence was found (Devaux *et al.*, 2021).

It is seen that susceptibility of children and adolescents aged from 0-19 are relatively poor in number and approximately it was near around 2%, as

per the data published by the Chinese Center of Disease Control and Prevention (Lu *et al.*, 2020; Wu & McGoogan, 2020). The possible reason can be due to differences in the development, distribution and function of ACE2 protein pattern in children (Lu *et al.*, 2020; Wu & McGoogan, 2020). This virus is connected to a reduction in CD4 cells (%) in older men relatively than in women and young men (Zhu *et al.*, 2020a). Some studies showed that children have increased numbers of CD4 cells and lower numbers of CD8, T lymphocytes than in adults (Zhu *et al.*, 2020a). Also, children and adolescents have less exposure to outside due to school closure and their underlying comorbid conditions are low (Lu *et al.*, 2020). However, the burden on children is increasing with time (Lu *et al.*, 2020).

At present, there is not any concrete information available that pregnant women are more susceptible to the disease and severe adverse outcomes of COVID-19 compared with the general population. However, during pregnancy women become immunosuppressed and other body chemicals get changed such as oxygen consumption getting higher, T lymphocytic immunity changes and diaphragm raises which can lead to increased risk of respiratory disease (Wei *et al.*, 2020). Another study shows susceptibility of occurrence among different blood groups. However, this study needs further investigation as it was a premature study. The study shows that patients with blood group O are low in number meaning the lesser risk of occurrence of COVID-19 in the O blood group. On the other hand, people of the A blood group were more in number meaning higher risk (Zhao, 2021)

In another study it was seen that not any significant changes in ACE2 expression found in different races such as Asian and white people, male and female or sub ages or younger than 60 (Cai, 2020). However, it was high in Asian smokers than non-smokers Asian (Cai, 2020).

Diagnosis

Incubation period: Novel coronavirus (SARS-CoV-2) shows specific incubation period ranging

from 2 to 14 days; howbeit often it also varies from 3 to 7 days (Chen *et al.*, 2020).

Sampling: According to Centers for Disease Control and Prevention (CDC) and WHO (CDC, 2020), specimens can be collected in different ways and these are upper respiratory specimens known as nasopharyngeal (NP), oropharyngeal (OP) swab or wash in ambulatory patients, a nasal mid-turbinate swab, an anterior nares (nasal swab) specimen swab, a nasopharyngeal wash/aspirate or nasal wash/aspirate (NW), lower respiratory specimens such as sputum (if produced), endotracheal aspirate and broncho alveolar lavage (in patients with more severe respiratory disease (Interim Guidelines for Clinical Specimens for COVID-19 testing, 2020). Storage temperature of the sample is 2-8°C for up to 3 days after collection (Interim Guidelines for Clinical Specimens for COVID-19, 2020). If there is a possibility of delay in testing or shipping, this temperature will be -70°C or below (Padula, 2020).

Who should get tested?

As suggested by the Centers for Disease Control and Prevention (CDC), not everyone is needed to get tested for COVID-19 (Testing for COVID-19, 2020). A guideline has been prepared for who should be tested; however, decisions of testing can be varied with assigned clinicians' on-spot experience about individual cases (Symptoms of Coronavirus, 2020) in such affairs, where two factors authentication is considered. Firstly, the patient's signs and symptoms are likeliness to COVID-19 and secondly, epidemiological factors mean the prevalence of community transmission in a specific jurisdiction. CDC has prepared a three levelled 'Priority List'. In PRIORITY 1, optimal care options for all hospitalized patients, lessening the risk of nosocomial infections and maintenance of the integrity of the healthcare system are ensured. People who are most at risk for infection complications are swiftly identified in PRIORITY 2. Testing community members for fast rising hospital cases is PRIORITY 3's focus in order to stop the spread of the disease and protect the wellbeing of integrated workers. Individuals without symptoms fall under NON-

PRIORITY list (COVID-19 Testing: What You Need to Know, 2020)

What to do after test result?

If the test result comes out positive for COVID-19, then further separation and treatment protocol have to be followed under the clinician's advice and monitoring (COVID-19 Testing: What You Need to Know, 2020). If test is found negative that does not guarantee that he or she will not get sick. Rather it indicates that the patient was not infected at time of specimen collection and was in very premature infectious stage and can get infected and develops the disease later on (COVID-19 Testing: What You Need to Know, 2020).

When to seek medical attention?

If any patient experience extreme symptoms of COVID-19 such as difficulty in breathing, pressure in the chest or persistent pain, bluish lips or face, inability to arouse, confusion, etc., then he/she should seek medical attention immediately (COVID-19 Testing: What You Need to Know, 2020).

Clinical symptoms: SARS CoV upshots influenza-like symptoms including fever, malaise, myalgia, headache, diarrhea, and shivering (rigors) (SARS (Severe Acute Respiratory Syndrome), 2020). Fever is the most prominently used tool to diagnose this disease, howbeit in elder people and immune-compromised patients fever remains under state at the initial stage. According to World Health Organization (WHO), initially, at first and /or during the second week of illness, dry cough which turns into wet later on, shortness of breath and diarrhea exist. In severe cases, rapid and continuing respiratory distress can be present and demand intensive care service immediately (SARS (Severe Acute Respiratory Syndrome), 2020). The common clinical symptoms of SARS-CoV-2 are not specific to individuals (Zu *et al.*, 2020). The most common symptoms are fever, cough and myalgia or fatigue (Israfil *et al.*, 2021; Zu *et al.*, 2020). These symptoms can be accompanied by nasal congestion, expectoration, cyanosis, runny nose and headache (Zu *et al.*, 2020; Chen *et al.*, 2020). In the pediatric patient, low to moderate and

sometimes even, no fever is observed (Chen *et al.*, 2020). Initially, some patients were found to have diarrhea and nausea before feverish appeared (Zu *et al.*, 2020). Overall fever is found dominant. However, few patients can have a headache or hemoptysis (Guan *et al.*, 2020b; Wang *et al.*, 2020b). Some can be asymptomatic (Gao *et al.*, 2020) especially the children. For the older patient with comorbidities, respiratory failure can be in offing due to severe alveolar damage in the lungs (Chen *et al.*, 2020). In extreme cases, one set of diseases can show organ failure or dysfunction rapidly such as shock, acute respiratory distress (ARDS) syndrome, acute cardiac injury, acute kidney injury which ultimately can lead to death (Wang *et al.*, 2020; Huang *et al.*, 2020a). NanShan Zhong's team's study reveals that the most regular symptoms were fever (87.9%), cough (67.7%), diarrhea (3.7%) and vomiting (5.0%) (Guan *et al.*, 2020), and 25.2% of the patients had either hypertension or chronic obstructive pulmonary disease. In the intervening time, hematological reports can show normal or lower white blood cell counts, lymphopenia or thrombocytopenia (Terpos *et al.*, 2020; Chen *et al.*, 2020; Huang *et al.*, 2020). Thromboplastin time can be extended and C- reactive protein levels can get higher (Terpos *et al.*, 2020; Chen *et al.*, 2020; Huang *et al.*, 2020).

Laboratory tests for the diagnosis of COVID-19

Molecular tests for the identification of COVID-19 by polymerase chain reaction (PCR): During the SARS outbreak, polymerase chain reaction (PCR) has been widely used among all other diagnostic tools.

Severe acute respiratory syndrome (SARS)-laboratory diagnostic tests: This method uses blood, stool, respiratory secretions or body tissue specimen to detect the genetic materials of the virus. In the critical situation of COVID-19, WHO made easy access to the Primers (a key element of PCR test). Later on, a ready-to-use PCR test kit was developed where both the primers and the positive and negative control were included. Suspected COVID-19 cases should be screened to test the presence or absence of virus by nucleic acid amplification tests (NAAT),

such as, rRT-PCR (real-time reverse-transcription polymerase chain reaction). The genes which are targeted here are N, E, S, and RdRP genes. RNA extraction must be done in a biosafety cabinet in a biosafety laboratory level-2 (BSL-2) or equivalent facility.

Chest X-ray for the diagnosis of lungs condition and pneumonia: Fang *et al.* (2020a) stated that the use of chest CT scan is better for the patients having clinical and epidemiologic features well-matched to COVID-19 infection especially when RT-PCR testing came out negative. The article also mentioned that for the early detection of the disease, non-contrast chest CT can be used. However, the viral nucleic acid finding process that means real-time polymerase chain reaction (RT-PCR) remains the standard of reference. Chung *et al.* (2020) described that chest CT may come out negative for viral pneumonia of COVID-19 at the primary stage (3/21 patients). On the contrary, Xie *et al.* (2020) stated that 3% of patients had negative RT-PCR for COVID-19 regardless of chest CT results typical of viral pneumonia.

Serological testing for the diagnosis of COVID-19: When NAAT assay comes out negative but strong epidemiological evidence navigate towards COVID-19 possibility, paired serum test can tell the truth. The WHO advises to do so validated serology tests, which is under development now (Bai *et al.*, 2010; Xiao *et al.*, 2020). However, it has been testified that the serum antibody ELISA and Q-PCR jointly can detect the early diagnosis of COVID-19 infection (Guo *et al.*, 2020; To *et al.*, 2020). Once the virus invades the body, in response to this virus (antigen), the body's natural defense mechanism produces antibodies, for example, IgM and IgG. Their level gets changed throughout the course of time. This test detects the level of these antibodies and thus detects the causative virus name. ELISA (enzyme linked immunosorbant assay) test can detect a mixture of two antibodies in the infected patient's serum nearly around day 21 after the onset period in suspected human cases. IFA (immunofluorescence assay) test determines the level and presence of IgM

are in the patient's serum. However, this test yields positive results approximately after day 10 of illness. In the case of the COVID-19 death study, it is stated that the CRP gets increased considerably after the onset and persisted for more than 14 days (Xu *et al.*, 2020a). The increase of S-IgG in non-ICU patients positively interrelated with the decrease of CRP (Xu *et al.*, 2020b).

Rapid diagnosis of COVID-19 by testing kit: The COVID-19 RNA kits which are permitted under EUA (emergency use authorization) went through a lesser time for development and regulatory review (Kapitula *et al.*, 2020). Thus, higher variability and quality issues come in front and results in false-negative results (Kapitula *et al.*, 2020).

Transmission

Symptomatic transmission: Symptomatic transmission of COVID-19 denotes the transmission from one individual with symptoms to another (Malik, 2020) and it is called the primary mode of transmission. It can happen due to maintaining close contact with the contaminated things or surfaces (Malik, 2020). Proof suggests that the very first three days are the days of the highest viral shedding period in upper tract (Malik, 2020).

Pre-symptomatic transmission: It results in just before the symptoms starts appearing (Malik, 2020). Evidence was also found in cases where infected individual can spread the disease even 1-3 days before their test result came positive, meaning the higher transmission capacity of virus even before they became symptomatic (Malik, 2020).

Asymptomatic transmission: During incubation period which approximately ranges from 5 to 6 days, transmission occurs and sometimes extends up to 14 days (Malik, 2020).

Human-to-human transmission: There are several ways for the transmission of COVID-19 viruses from human to human. One possibility is through aerosol transmission (Yang *et al.*, 2020; Zu *et al.*, 2020). Through droplets, contact and fomite, human-to-human transmission has been confirmed by the cluster of some infected family members and

from one person to another individual who infected another individual following a chain of four generations (Lam *et al.*, 2020; Zhang *et al.*, 2020). An article stated that in the feces of confirmed patients this virus was found. This indicates that may be it can replicate in the digestive organ as well and from this fecal to the oral transmission can also occur (Yang *et al.*, 2020). SARS-CoV-2 can persist in the air for several hours in aerosols, can be spotted up to 3 hours after aerosolization and can communicate a disease to cells by this time (Sharma, 2020).

Animal to human transmission: Coronaviruses generally infect birds and animals (Sharma, 2020). However, some successfully crossed this barrier and progressively formed zoonotic diseases in humans (Sharma, 2020).

Prevention of transmission COVID-19

Preventive measures for general people: The best way of prevention is to maintain a strategic distance from being exposed to the infection. A number of defensive strategies, including airborne protections, have been suggested. By using a face mask, covering coughs and sneezes with a bent elbow, washing and sanitizing hands and open surfaces, using sanitizer with at least 60 percent alcohol and avoiding touching your eyes, nose, or mouth with unclean hands, infection prevention and control (IPC) measures can reduce the likelihood of exposure and dodge social gathering as much as possible.

Preventive measures in the healthcare centre: Healthcare providers are at the highest risk. For their safety, they should be provided with hand sanitizers, hand gloves, masks and other PPEs (personal protective equipments). Suspected people should be sent to a private room or negative pressure room if available (Chang *et al.*, 2020; Radonovich *et al.*, 2019; Cowling *et al.*, 2009).

No discernible difference was identified between the incidences of laboratory-confirmed influenza among healthcare workers using N95 mask respirators (8.2%) vs. surgical masks in a randomized clinical trial involving 2862 medical professionals (7.2%) (Radonovich *et al.*, 2019). If an affected

patient admits into the hospital and an airborne infection isolation room is not available, the patient should transfer to an isolated area to minimize the contact of health care providers and other patients (Raboud *et al.*, 2010). As it is not confirmed how long the virus stays on the air after a patient leaves, that room needs respiratory protection for a period which is definite to the ventilation and clearance rates for the room (Yee *et al.*, 2020).

Community-based preventive measures: Zhang *et al.* (2020) expressed that individuals can take a few preventive measures such as populace portability control to cut the transmission, gathering control, closing down cinemas, prayer halls, entertainment parks, restaurants, etc. (Zhang *et al.*, 2020). It makes a difference to avoid the infection from the huge transmission. Community framework administration by a computerized strategy will be a great way to control the transmission among the mass individuals (Zhang *et al.*, 2020).

General precautions to prevent contamination: People who have had suspect exposure and close contact should undergo a 14-day isolated health observation. The final day of interaction with SARS-CoV-2-infected patients or an unsettling atmosphere

would mark the beginning of this isolation (Sohrabi *et al.*, 2020). Based on the projected SARS-CoV-2 incubation time, the World Health Organization (WHO) and the Centres for Disease Control and Prevention (CDC) in the United States advise 14-day quarantine for people who had close contact with a confirmed case. Tests have shown that COVID-19 can persist in the smallest of these droplets, known as aerosols, for several hours (van Doremalen *et al.*, 2020). In fact, there have been cases of widespread infections being spread by a large number of people in confined spaces. Wearing a mask is essential for limiting the transmission of COVID-19 through droplets (Zhou *et al.*, 2020), as per the Director General of the Chinese Centre for Disease Control and Prevention (CDC). According to Ma's research, N95 masks, medical masks and even homemade masks may be able to block at least 90% of the virus in aerosols (Ma *et al.*, 2020).

Individuals who have come to the near contacts and suspicious environment, got to be prompted to have a 14-day of quarantine (Sohrabi *et al.*, 2020) as recommended by WHO and CDC. The summarized preventive measures against COVID-19 have been presented in figure 5.

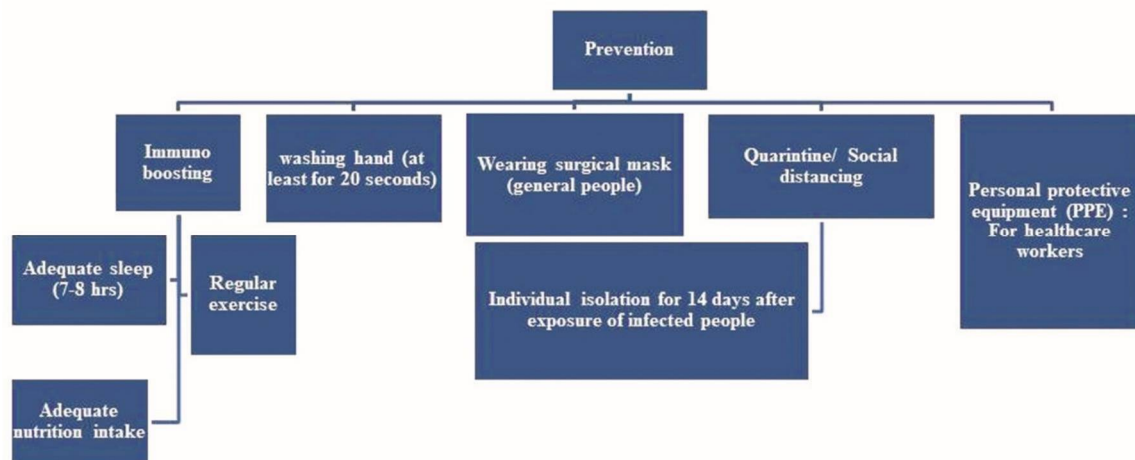


Figure 5. Preventive measures against COVID-19.

Boosting immunity: Proper hygiene, balanced diets, regular physical exercise, adequate sleep & rest and boosting immunity are some important measures that not only help to prevent COVID-19 infection but

also help to maintain emotional stability and mental health (Shen *et al.*, 2020).

Vaccination for the prevention of COVID-19 infections: According to the information reported by

WHO, a total of 1,953,935 confirmed case of COVID-19 with 29,131 deaths has been reported in Bangladesh from 3rd January 2020 to 10 June 2022 (WHO, 2022). The most effective measure for the prevention and attenuation of the infections of COVID-19 viruses is vaccination (Bari et al, 2021). After tremendous and restless efforts of the scientists all over the world, several COVID-19 vaccines have been approved by the regulatory authorities. As of the most recent update on 10 June 2022, a regulatory authority, a national authority, or another organization had approved, authorized, licensed, granted emergency use authorization to, or made accessible for use outside of clinical trials, a total of 38 vaccines (COVID-19 vaccine tracker, 2022). This covers 197 countries with approved vaccines.

In Bangladesh, the first vaccination started as pilot vaccination program on 27 January 2021 and mass vaccination started on 7th February 2021. Eight vaccines approved for use in Bangladesh named Covovax (protein subunit) by Serum Institute of India, Spikevax (RNA vaccine) by Moderna of United States, Comirnaty (RNA vaccine) by Pfizer/BioNTech, Sputnik V (Non-Replicating Viral Vector) by Gamaleya, Ad26.COVS.2.S (Non-Replicating Viral Vector) by Johnson & Johnson, Covishield (Oxford/AstraZeneca formulation) - non-replicating viral vector vaccine by Serum Institute of India, Covilo (inactivated) by Sinopharm (Beining), and Coronavac (Inactivated) by Sinovac (China) (COVID-19 Vaccine Tracker, Bangladesh, 2022). As on 11 June 2022, total people registered for vaccination is 99,637,388 (83.6% against population), 1st dose of vaccine administered 128,854,793 (75.66% against population), 2nd dose of vaccine administered 118,183,983 (69.39% against population), and 3rd dose administered 24,037,571 (20.34% against 2nd dose) (COVID-19 vaccination Dashboard for Bangladesh, 2022). Because of the successful progress of mass vaccination in Bangladesh, the rate of COVID-19 infection and number of deaths have been tremendously improved. As per the available information on the website of DG Health, Ministry of Health, Government of Bangladesh on 11 June 2022, in the last 24 hours

4755 suspected people were lab tested, among them 64 confirmed cases were identified with zero death (DG Health, 2022).

Conclusion

The unprecedented outbreak of COVID-19 is ongoing around the world and has been a threat to the human civilization. By gathering knowledge from 2003 incident of SARS-CoV-2 which was animal to human transmission, all the game plan to combat this pandemic has to be optimally regulated centrally. As it is expected that the world will see couple of more waves of this disease, it is highly necessary to follow instructions from being staying at home to maintaining social distancing and wearing face masks and shields. To do this, vigilant and strict control measures are needed to be followed globally. Furthermore, advanced intervention strategies are required to precisely predict the future. It is essential to isolate patients, after tracing them, quarantine as soon as possible and gives them mental support. It is also indispensable to educate the public regarding both food and personal hygiene and to make the health workers ready to act on compliance to control and mitigate the super spreading events. Globally, the COVID-19 pandemic is a major health concern. Everything protocol relates to this is at immature stage and constantly requires updating starting from its disease history, background, diagnosis protocol, preventive measures to regulatory protocol development. However, a strict protocol is needed to be maintained to avoid ending up under-rated articles getting published and misinformation is getting highlighted. However, the number of COVID-19 new cases drastically improved with vaccination and death rate has become zero in Bangladesh. The COVID-19 situation all over the world has improved with the implementation of vaccination. However, the new variants of coronaviruses are still a threat for the increase of new infections and require continuous efforts of the discovery of new vaccines and medications for the prevention and treatment of COVID-19. Still the future is not known to anyone, how many and when the new waves of serious

infections like COVID-19 will come and human needs to fight against it.

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Author contributions

MMRS conceptualized the study. SKS, FK, SL, MIS, TA, SA, KFC, UMTA, SI, KAM, and SA searched the databases and collected articles of this review. SKS, FK, and SL wrote the draft of the manuscript. MJH, MHR and MAR critically revised the manuscript. MMRS critically evaluated, revised the manuscript and supervised the whole project. SKS and FK edited the final manuscript as per reviewer comments.

Conflict of interest

The authors declare that they don't have any commercial or financial or intellectual conflict of interest.

Data availability statement

All data generated and/or analyzed in this study have been included in the article. Additional data or information will be provided on request by the corresponding author.

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