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Living with COVID-19: The Nemesis, the Hubris, and the Elpis

Subject Category: Clinical Virology

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Chapter 1: COVID-19: Exploring the Disease Transmission Dynamics

Background

The immuno-thrombo-inflammatory disease: COVID-19 is an acute immuno-thrombogenic inflammatory viral disease manifested by dysfunctions related to multiple organs involved in its pathogenesis. Its incidence and prevalence of is related to environmental viability of the virus, various transmission factors associated with the agent and the host, possible modes of transmission, period of infectiousness, and composition and susceptibility of the population. Whereas respiratory route is dominant mode of transmission, transmission through direct contact or fomite transmission do occur.

SARS-CoV-2: Understanding the agent factors: Understanding of SARS-CoV-2 or hCoV-19 structural components is important for understanding the dynamics of the disease transmission and propagation. The virus has a lipid shell, a single-stranded RNA genome containing 29891 nucleotides, spike, envelope, membrane and hemagglutinin-esterase dimer proteins. The SARS-CoV-2 structural components have been related to the COVID-19 pathogenic mechanisms.

The kinetics of transmission and propagation: Transmission of SARS-CoV-2 to cause COVID-19, requires that a minimum but unknown dose of replication-competent virus be delivered to an appropriate anatomical site in a susceptible and vulnerable host. A combination of various agent (viral), host, and environmental factors influence the transmission and course of the disease.

Fear, confusion, and impact on mass behaviour: The un-relented spread of COVID-19 pandemic is a major public health concern threatening general and mental health and safety of the human life all over the globe. The associated anxiety and emotional stress levels are often high. The healthcare professionals, too, suffer with various concerns like long and strenuous working hours, being able to provide competent medical care, their safety at the workplace and taking the infection home to their family, uncertainty that their organizational support for their personal and family needs.

Conclusion: **The lessons for pandemic control**: The human subjects produce respiratory droplets ranging from 0.1 to 1000 μm. Depending on droplet size, inertia, gravity, and evaporation factors, the emitted droplets and aerosols disperse in air. There are two obvious transmission pathways: the airborne inhalation and contracting through the contaminated surfaces. It appears that the contaminated surfaces may play less significant role as compared to the infected airborne droplets and the aerosol in the disease transmission.

The immuno-thrombo-inflammatory viral disease

The Virus and the Disease: The COVID-19 is an acute immuno-thrombogenic inflammatory viral disease manifested by dysfunctions related to multiple organs involved in its pathogenesis. Further, the incidence and prevalence of the disease have been related to the environmental viability of the virus, various transmission factors associated with the agent and the host, possible modes of transmission, period of infectiousness, and composition and susceptibility of the population [1]. Whereas the respiratory route is common and the dominant mode of transmission, transmission through direct contact or fomite transmission do occur. Other modes, such as, through sexual, fecal–oral, and bloodborne routes have not been documented. Similarly, there are no confirmed cases suggesting in-utero transmission or through breast-feeding or through domestic pets.

For the transmission through respiratory route, physical proximity is the key determinant with the densely packed and ill-ventilated spaces accentuating the probability of transmission. In the clinical setting, the transmissibility correlates with severity of symptoms, albeit the asymptomatic patients also transmit the infection complicating the disease epidemiology. The period of infectiousness has been documented to be about 9 days of symptomatic phase, though the detectable virus shedding may persist for longer periods [2]. In addition, it is also associated with the duration and frequency of exposure and linked to the contracted viral load.

COVID-19 Scenario in Developing Countries: Considering in a totality, the COVID-19 cases in developing countries are more than the developed countries. The high population density, smaller dwelling units, and lack of quality healthcare compounded with illiteracy and indifferent attitude of the people and government organisations have been related to a high-incidence setting accompanied with high morbidity and mortality. In the low-income countries, the reported cases

and deaths have been concentrated in younger cohorts than expected from observations in higher-income countries, even after accounting for demographic differences across settings. It has been noted that both cases and deaths due to the viral illness have been mostly concentrated in the 40-69 years age group in India as compared to what seen in high-income countries, among other trends [3].

Our current understanding of COVID-19 is largely derived from clinical and epidemiologic studies of the pandemic undertaken in in China and various European countries and North America. There are over 1.3 billion people are at risk of SARS-CoV-2 infection in India. Further challenges exist for the healthcare providers in smaller cities and rural areas, as the COVID-19 pandemic has put a tremendous impact on primary care practices. The population groups and people often fail to comprehend that the pandemic is real. They are uncomfortable with the required prerequisites for social interaction, prevailing uncertainty about their health, economic issues, and various other reasons.

SARS-CoV-2: Understanding the agent factors

Understanding of SARS-CoV-2 or hCoV-19 structural Components is important for understanding the dynamics of the disease transmission and propagation. The virus has a round, elliptic or pleomorphic shape with a diameter of 60–140 nm. It has a single-stranded RNA genome containing 29891 nucleotides, encoding for 9860 amino acids and bearing a sequence identity approximately 50% to MERS-CoV and 79% to SARS-CoV. Like other CoVs, it is sensitive to ultraviolet rays and heat and inactivated by lipid solvents including ether and ethanol.

The SARS-CoV-2 virus has a lipid shell, an RNA genome, spike, and envelope and membrane and hemagglutinin-esterase dimer proteins (Figure 1). The SARS-CoV-2 structural components have been related to the COVID-19 pathogenic mechanisms.

1. The glycosylated Spike protein (S) forms the distinct spikes on the surface of the virus. It utilizes an N-terminal signal sequence to gain access to the endoplasmic reticulum and mediates the attachment to host angiotensin-converting enzyme II (ACE2) receptors. The S protein is cleaved by a host cell furin-like protease into two separate polypeptides S1 and S2. The D614G mutation is thought to increase the transmissibility of the virus through enhancing the binding of the S protein to ACE2.

2. The viral RNA genome is bound by the phosphorylated nucleocapsid protein (N) in a beads-on-a-string conformation. On entering the host cells, the N protein potentially tethers the viral genome to replicase-transcriptase complex (RTC). It also helps in packaging the encapsulated genome into viral particles.

3. The envelope (E) protein is found in small quantities and appears to have be a transmembrane ion channel activity. The protein facilitates assembly and release of the new virions. It is related to the disease pathogenesis and important for the disease progression.

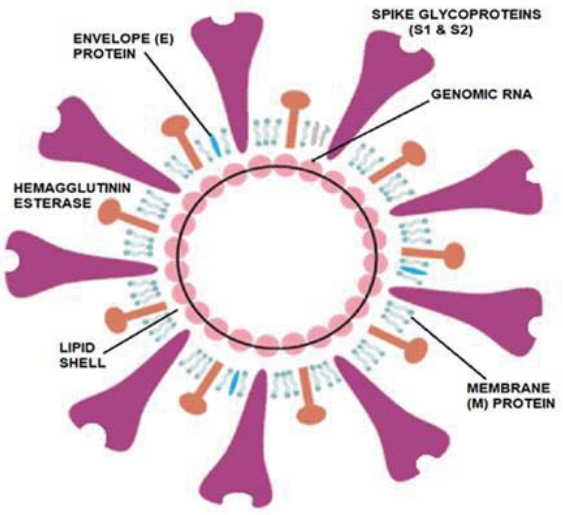

Figure 1: The structure and components of COVID-19 Virus.

4. The Membrane protein (M) is the most abundant structural component of the virus. It exists as a dimer and enables to maintain membrane curvature on one end and bound to nucleocapsid proteins on the other.

5. The Hemagglutinin-esterase (HE) is also a dimer protein and binds to sialic acids on surface glycoproteins. It is responsible for facilitating and enhanceing S protein-mediated cell entry and virus spread through the host mucosal cells.

6. SARS-CoV-2 uses a methyltransferase to cap its messenger RNAs to prevent them from being recognized by the host immune system and ensure their translation in host cells [4]. Disrupting the formation of the active methyltransferase complex and/or block its catalytic activity appear to be a potential strategy for developing COVID-19 therapeutics.

Dynamics of transmission and propagation

Transmission of SARS-CoV-2 to cause COVID-19, requires that a minimum but unknown dose of replication-competent virus be delivered to an appropriate anatomical site in a susceptible and vulnerable host. A combination of agent (viral), host, and environmental characteristics influence the transmission. Live virus has been isolated for up to 3 hours from aerosols and variable period from various surfaces. The virus is able to persist on plastics and stainless-steel surfaces for longer periods, with the half-life being about 6 hours. In the real-world settings, though the live virus is not detectable on most high-touch surfaces. The virus is stable at low temperatures but sensitive to heat. Various disinfectants may denature the virus.

New insights into COVID-19 viral spread:

1. Whereas there is a rising trend in the infection rise as people are returning to their work-places, in other places the outbreaks are related to the younger people crowding and partying care-freely and other people trying to resort back to their earlier ways of life. Because most of the new outbreaks involve the cases in younger age groups, fewer of them develop severe form of the disease. But it is only a matter of time before the elderly people are affected leading to higher mortality. Further, reopening the educational facilities across the continents may make matters worse.

2. The airborne transmission: The transmissibility of SARS-CoV-2 is high. The infected individuals release aerosols and droplets containing SARS-CoV-2 while talking, coughing, or sneezing. These virus-containing aerosols and droplets can lead to short-range airborne transmission (~ 6 ft). Much depends on the size of the aerosols (< 10-µm diameter) and droplets (> 10 µm diameter) which can promote infection through direct inhalation and deposition on surfaces and subsequent hand-to-mouth/nose/eye transfer (Figure 2).

The suspended airborne droplets appear to persist in the air for several minutes. Whereas the smaller aerosols can persist for longer durations (several minutes to hours). The characteristics of aerosols are dynamic, due to evaporative loss of water depending on ventilation, humidity, and temperature levels. With decreasing size, their ability to disperse in air is enhanced, leading to their transmission extending beyond 6 feet from the point of release.

3. The aerosol size fractions may influence the deposition profile of SARS-CoV-2 in the lung [5]. Thus, whereas

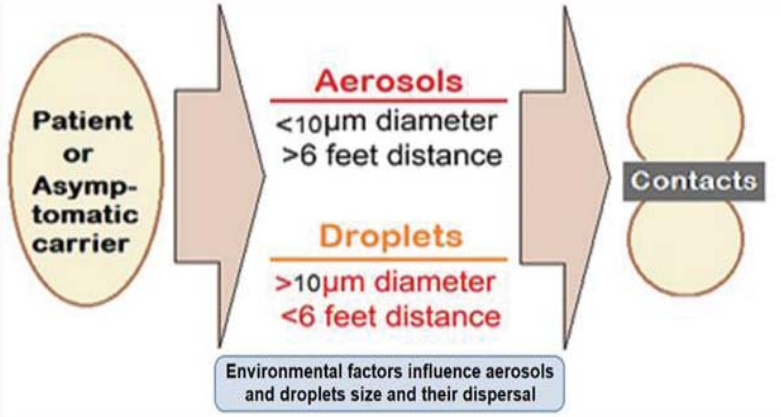

Figure 2: SARS-CoV-2 transmission dynamics - Droplets and aerosol concepts.

the larger aerosols (> 4 µm) are predominantly deposited in the upper and central airways (i.e., nasopharynx, tracheobronchial) and are subject to muco-ciliary clearance, the smaller aerosols (< 4 µm) get deposited deeper in the alveoli, having epithelial cells rich in ACE2, with enhanced transmission efficiency.

4. The transmission may occur through fomites also but appears to play a minor role (Figure 3). Further, the emphasis on hand hygiene has been diluted, as it is becoming clear that contaminated surfaces may not play a large role. Similarly, the emphasis on banning outdoor activities is losing focus, as it is becoming established that the outdoor activities like jogging, outdoor hospitality, non-essential shopping, and public transportation are fine as long as people keep social distancing and wear face masks. The focus is, rather on indoor activities which are the main culprit for the virus transmission.

5. The emphasis should also be on targeting outbreak clusters and super-spreading events. The studies indicate that 10 percent of patients cause 80 percent of all the infections, whereas most patients to the tune of 90 percent do not infect further. As such, the superspreading events are uncommon [6].

The backward contact tracing is more useful than the forward tracing. Further, finding clusters help epidemiologists in understanding about the outbreaks. With more stress on the long-term care facilities and workplaces, the specifically targeted measures help in preventing outbreaks, rather than general lockdown measures. These targeted versions include encouraging people to work from home and avoiding crowded places and banning meetings and gatherings.

6. Using the preventive measures and resources rationally, various countries are now better equipped than before in the current scenario. We are aware of the disease epidemiology, the virus transmission kinetics, and equipped with resources like PPE kits and masks. Further, the rational behaviour has emerged in place of irrational fears about the disease. In addition, most countries have developed machinery for contact tracing, surveillance and gathering data.

The viral transmission and propagation: With the infection contracted, the viral load is highest in the upper respiratory tract (nasopharynx and oropharynx) early in disease and then increases in the lower respiratory tract. Susceptibility to SARS-CoV-2 infection increases with age; children < 10 years (4% - 7%) are around half as susceptible as adults (14% - 17%).

As with other respiratory infections, the transmission occurs through droplets discharged by the infected person or by being carried to oral or nasal mucosa by hands from the virus-infested surfaces. It appears that all COVID-19 patients – asymptomatic, mild, or severe shed a massive number of virus in the surroundings. Aerosol transmission may also be possible in closed and confined spaces. The incubation period is from 3 to 7 days, ad 14 days is being considered as the longest possible time from infection to appearance of symptoms. In addition to the respiratory droplets and direct contact, fecal–oral transmission may also be a route of transmission. It is to be cautioned that the information is derived from the early research and reports and further studies may aid understanding the mechanisms of transmission, the incubation period, duration of infectivity and the clinical course of COVID-19.

Steps in progression of COVID-19 illness: Once inside the airways, the virus through its surface (S) protein tends

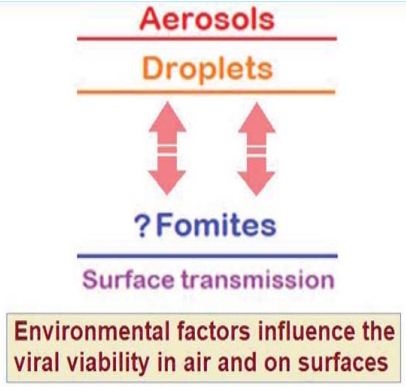

Figure 3: Transmission kinetics – Aerosols, droplets and fomites interlink.

to recognize and stick to the receptor protein called ACE2 and attack the ACE2-bearing cells lining the airways. It can infect upper as well as lower respiratory tracts and with the dying cells sloughing down and filling the airways the virus is carried deeper into the lungs. The thin layer of surfactant coating the airways becomes even thinner and the brush border less efficient to evict viruses and other foreign particles with a colder temperature and dry air, which may also dampen the immune response to the invading viruses. It appears that the virus is able to transmits while still confined to the upper airways, before invading the lower respiratory tract and lungs and causing severe symptoms. In addition, the virus is able to infect ACE2-bearing cells in other organs, including the blood vessels, gut and kidneys.

With the viral infestation, the activated immune system leads to macrophages to be recruited to the alveolar space, which increase cytokine production and attract additional immune cells such as T-helper cells CD4 and CD8 to the affected areas to combat the virus. The pattern recognition receptors (PRRs) of the immune cells recognize the virus and signal release of the pro-inflammatory cytokines such as interferon gamma (IFN-g), tumor necrosis factors (TNFs), interleukins (ILs), and chemokines. IFN-g activates macrophages which produce IL-6, TNF-α, and IL-10. There are triggered additional pathways associated with PRRs, including cyclo-oxygenase (COX)-2 and c-Jun N-terminal kinase (JNK). With recovery, once the virus is cleared, the immune pathways shut down. However, the process goes into overdrive in a cytokine storm, initiating vascular leakage, coagulation cascades, and disseminated intravascular coagulation (DIC). Further, it leads to an increased susceptibility to infectious bacteria. Furthermore, the process affect other organs besides the lungs, especially in those suffering chronic diseases, leading to multi-organ failure (MOF).

Age is an important epidemiological factor. The elderly people are at risk of severe infections possibly because the ineffective initial anti-viral immune response. It appears that children may be less severely affected because their immune system is unlikely to progress to a cytokine storm. Further, there are other factors like individual genetic make-up, frequency of exposure, the amount of virus load, the other microbes in the body including gut microbiota which may play a role in acquirement of the infection and its progression.

Directed by the viral RNA, the synthesis of polyprotein 1a/1ab (pp1a/pp1ab) in the host cells is brought about with the transcription occurring through the replication-transcription (RT) complex organized in double-membrane vesicles (Figure 4).

The open reading frames (ORFs) guide the production of both pp1a and pp1ab polypeptides which are processed by virally encoded chymotrypsin-like protease (3CLpro) or main protease (Mpro), as well as one or more papain-like proteases for producing 16 non-structural proteins (nsps). The ORFs also encode for structural proteins, including spike, membrane, envelope and nucleocapsid proteins. The pathophysiology and virulence mechanisms of COVID-19 virus is related to the function of the nsps and structural proteins. Further, the nsps appear to block the host innate immune response, as well. The E protein also plays a crucial role in in promoting viral assembly and release of nascent virions.

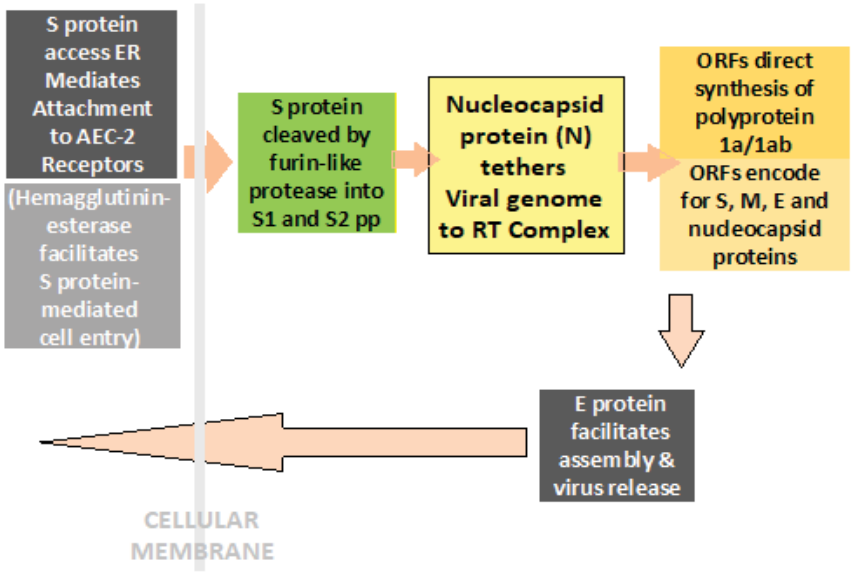

Figure 4: Simplified Steps in COVID-19 Pathogenesis and Replication Cycle.

The role of ACE2 receptors in COVID-19: It appears that the viral spike (S) protein plays an important role in viral attachment, fusion, and entry. ACE2 being the main host cell receptors for, plays a crucial role in the invasion of virus into the cell through the receptor binding domain (RBD) and infectivity and affliction of upper respiratory tract, lower respiratory tract and lungs, and gastrointestinal system apart from kidneys and various other organs. Considering this, the expression and distribution of the ACE2 in human body may outline the potential infection routes and the organs with high ACE2-expressing tissues are potential sites for 2019-nCoV infection. Further, the ACE2 expression is reduced in SARS-CoV infection and has been linked with the disease manifestations. In mice experiments, the injection of SARS-CoV Spike worsens acute lung failure *in vivo*, which can be attenuated by blocking the renin-angiotensin pathway [7]. A related issue is that of ACE2 Receptor Polymorphism, which may influence the infectivity as well as disease severity following exposure to SARS-CoV-2 virus [8].

Significantly high ACE2 expression is found in type II alveolar cells (AT2) of lung, upper oesophageal stratified epithelial cells, absorptive enterocytes from ileum and colon, gall bladder and bile ductal cells, myocardial cells, kidney proximal tubule cells and bladder urothelial cells. In the lungs, the bulk of the ACE2-expressing cells, over 80 percent, are AT2 (alveolar type 2) cells. The abundant expression of ACE2 in the AT2 cells may explain the preferential involvement of lungs and severe alveolar damage following the infection. The distribution of ACE2 is more widespread in males than females, which is consistent with the epidemiological finding that men are more prone to COVID-19 than women. The ACE2 expressing of ACE2 AT2 cells also express many other genes favouring the viral processes. The ACE2 is also expressed on the oral mucosa and highly enriched in epithelial cells of tongue accounting for a potentially high risk of this route for 2019-nCoV infectious susceptibility. These findings may underline the prophylactic role of frequent mouth washing and rinsing [9]. Further, the saliva, urine and stool specimens and rectal swabs have demonstrated embedded viruses in the COVID-19 patients.

The multiple strains of SARS-CoV-2: The SARS-CoV-2 virus is mutating due to factors related to a neutral drift and random genetic changes. Initially, the virus which emerged from Wuhan was the D-strain. Currently, there are six strains of coronavirus. The original one is the D or L strain that appeared in Wuhan in December 2019. Its first mutation -- the S strain -- appeared at the beginning of 2020, while, since mid-January 2020, we have had strains V and G. To date strain G is the most widespread: it mutated into strains GR and GH at the end of February 2020. In North America, the most widespread strain is GH, while in South America, it is the GR strain. Globally and in Asia the strains G, GH and GR are progressively increasing and by far the most widespread, representing about three-fourth of all [10]. They present four mutations, having changed sequence of the RNA polymerase and Spike proteins of the virus. The strain S is found in some restricted areas in the US and Spain and the L and V strains are gradually disappearing.

As now, in total, there have been identified over 285 mutations in SARS-CoV-2. But as such the mutations do not seem to have any impact on the disease severity. Though, the new G-strain, which is increasingly affecting populations of various countries, seems to be more contagious [11]. The new G-strain, evolved as the result of a mutation known as D614G, has gradually replaced the D strain all over the world and now accounts for over 85% of published SARS-CoV-2 genomes [12]. In the new G-strain there is supplementation of aspartic acid (D) in the 614th position of the amino acid with glycine (G), hence, named as D614G. The D614G mutation which has occurred in the spike protein of the virus, helps the virus in attaching more efficiently with the ACE2 receptor in the human host and thus appears to have increased infectivity. This mutation was first identified in China and afterwards in Europe, has spread to various countries including the U.S., Canada, and India.

Long, et al. sequenced the genomes of 5,085 SARS-CoV-2 strains causing COVID-19 waves in an ethnically diverse population of Houston in the USA. They concluded that most of the strains in the second wave was having a Gly614 amino acid replacement in the spike protein, which could be linked to increased transmission and infectivity [12]. The findings also help in delineating the origin and trajectory of subsequent infection waves. In addition, among various SARS-CoV-2 variants detected, only a small proportion are of public health concern by virtue of being more transmissible, able to cause more severe illness, or can elude the immune response following infection and possibly from vaccination [13].

The second and subsequent waves of the pandemic are also characterized by SARS-CoV-2 strains with diverse genotypes. Virtually all cases in the second and ongoing disease wave had been caused by strains with the Gly614 variant of spike protein. In the Gly614 variant, amino acid residue Asp614 is located at subdomain 2 (SD-2) of the spike protein and forms a hydrogen bond and electrostatic interaction with two adjacent residues in the S2 subunit. The replacement of aspartate with glycine possibly substantively weakens the bond between the S1 and S2 subunits, producing a highly

fusogenic spike protein increasing the ability of S1 to dissociate from S2 and thus enhancing the Gly614 variant's ability to invade and enter the host cells. These findings highlight the importance of multiple importation events in the genetically diverse strains in epidemiology and pathogenesis of COVID-19.

Fear, confusion, and impact on mass behaviour

The COVID-19 situation, distress and behavioural effect: In most countries, fear levels were stated to rise in response to the mounting case count and governmental efforts to contain the pandemic through lockdown measures, over the last few months. The lockdown measures in general, have generated fears about the availability of essential goods and access to various amenities. The survey also found that while approving of the government's lockdown strategy, a significant number of citizens among various nations including Indians were fearful of the impact of lockdown on availability and quality of the essential services.

The spread of COVID-19 pandemic is a major public health concern threatening general and mental health and safety of the human life all over the globe [14]. There are a number of studies which have for assessed and validated of the anxiety and other psychiatric symptoms related to COVID-19 [15]. Another study involving general population, found that the anxiety levels and emotional stress were high, in general [16].

Reaction to COVID-19 among healthcare professionals: Assessment of the change in behavior and attitudes focusing on the healthcare providers' concern about getting infected and other related stress factors in context of the COVID-19 pandemic have been assessed in various studies. In general, there is a large concern about the aerosolized spread of the virus through being exposed to coughing respiratory patients and aerosol-generating procedures, associated with the apprehension that they may not be able to maintain a safe social distance appropriately. In addition, the anxiety and stress may also be caused by organizational factors and fear about resource shortages such as personal protective equipment (PPE) and protective masks are common, and concerns about not being able to provide competent care if deployed to a new area, about rapidly changing information and lack of communication, lack of specific drugs, the shortage of ventilators and intensive care unit beds necessary to take care for the surge of critically ill patients, and significant change in their own daily social and family life [17].

The healthcare professionals suffer with various concerns, which are different from the public at large. The responses from 8 listening sessions with groups of physicians, nurses, advanced practice clinicians, residents, and fellows have disclosed the sources of anxiety and concerns among healthcare workers. Hear me, protect me, prepare me, support me and care for me was the message from health care professionals dealing with the COVID-19 pandemic delivered to their organizations, according to an article published in JAMA [18]. On the part of the policy planners, along with maintaining critical supplies, there should be maintenance of an adequate healthcare workforce to maximize the ability of each healthcare worker to handle increased number patients and overall workload. The simple and genuine expressions of gratitude for the commitment of health care professionals, should be properly supplemented by concrete support and facilities.

In general, the healthcare professionals are worried about their safety at the workplace and taking the infection home to their family, uncertainty that their organization will support for their personal and family needs if they develop infection, and issues about strenuous duties and long working hours and being able to provide competent medical care and access to up-to-date information and communication [19].

Age groups and the perceived threat to COVID-19: Generations or the age-groups are often considered by their periodic span along the timeline, though there is no agreed upon formula to define the length of the span. In general, those born between 1981 to 2000 can be called Millennials (witnessed the dawn of the Millennium) or Generation Y. The Generation X is born between 1961 to 1980, whereas the Baby Boomers, Generation O, are those born between 1941 to 1960. Those born between 1921 to 1940 form the Silent or Generation Alpha. Whereas Generation Z (or Gen Z for short, or Zoomers) is the demographic cohort succeeding Millennials or Gen Y. Most members of Generation Z are born in the mid-to-late 1990s and the early 2010s, and most of the Gen Z have used digital technology since relatively young age and are comfortable with the Internet and social media and are the successors of Gen Y [20].

Through the collected data, in the survey by YouGov and the Institute of Global Health Innovation, it has been inferred that among the population groups within India and elsewhere, it is millennials or Gen Y who appear to be most fearful to COVID-19 infection compared to other age-groups. Among the millennials in India, women were more scared of the

infection than men [21]. Across Generations, those belonging to the Gen Z generation (post-millennial adults) were less scared than millennials. Even the older generations (Gen X and older, Gen Alpha), who are more vulnerable to COVID-19 than millennials, were less afraid of the virus and the disease than millennials (Figure 5).

Rational behaviour for COVID-19 prophylaxis: As regards the fear to contracting the virus, respondents in Asian countries have reported taking more precautions (such as avoiding public places and wearing face masks) compared to Western peers. On most parameters, Indians ranked close to the median. The survey also noted that in case of personal hygiene (washing hands, using sanitizers, etc.), people from some countries were relatively less cautious about hygiene compared to others from more developed countries. The residents from most of the counties, rated their governments' actions to contain the pandemic favourably. As per the survey, more than 80 percent respondents in Vietnam, India, and Denmark approved their government's response to handling COVID-19.

There are various surveys in offing and underway. The COVID-19 Symptoms & Social Distancing Web Survey by Harvard, aims to gather information on the prevalence of COVID-19 symptoms and social distancing behavior. Another survey, the Knowledge, Attitude and Practice Survey on COVID-19 is a platform to receive feedback from the public on measures to improve efforts in the fight against COVID-19 and encourage behaviour change. There is also an International Survey on Coronavirus COVID-19-survey.org to measure Worldwide COVID-19 Attitudes and Beliefs.

Conclusion: The lessons for pandemic control

The human subjects produce respiratory droplets ranging from 0.1 to 1000 μm. Depending on droplet size, inertia, gravity, and evaporation factors, the emitted droplets and aerosols disperse in air. The large respiratory droplets will undergo gravitational settling faster than the smaller droplets and aerosols, which are likely to be buoyant and transported by air currents. The COVID-19 infection occurs through the transmission of virus-containing droplets (> 5 to 10 μm) and aerosols (≤ 5 μm) exhaled from infected individuals during breathing, speaking, coughing, and sneezing [22].

The aerosols can accumulate, remain infectious in indoor air for variable period depending on various environmental conditions. Thus, there are two obvious transmission pathways: the airborne inhalation and contracting through the contaminated surfaces. As appears, the contaminated surfaces may play less significant role in the disease transmission compared to the infected airborne droplets. Thus, the aerosol transmission is the major factor in the spread of COVID-19 infection [23]. Further, aerosols can remain airborne for hours, accumulate over time, and follow airflows over distances more than 6 feet [24]. The SARS-CoV-2 virions may be contained in submicron aerosols and can remain airborne for prolonged periods. Further, in a closed space with asymptomatic individuals, infectious aerosol concentrations may

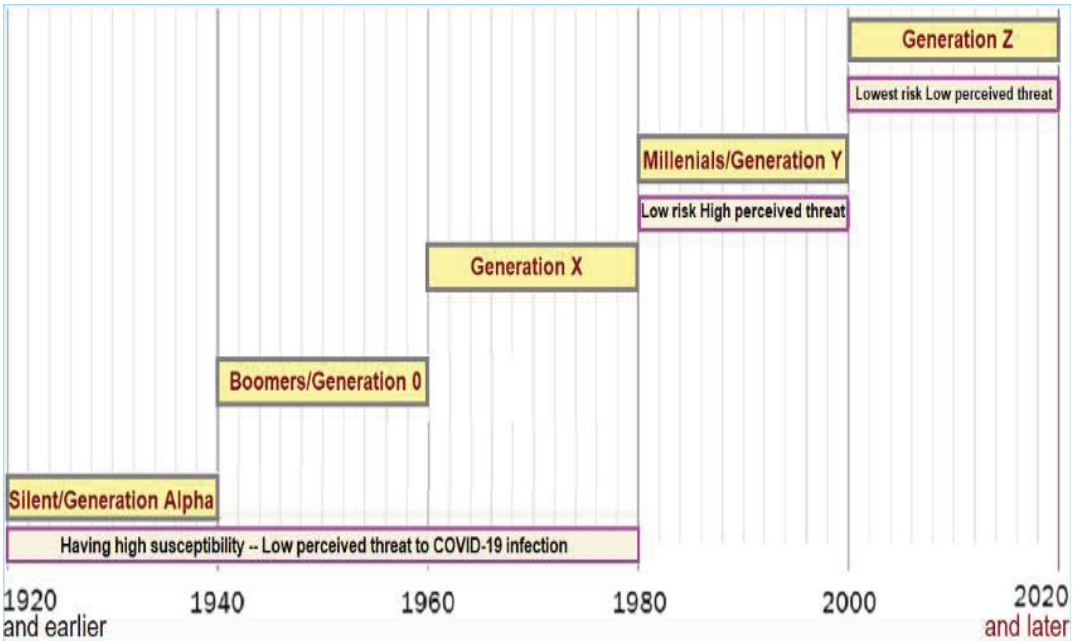

Figure 5: Age-groups and Generations Alpha, O, X, Y and Z and Risk and Perceived threat to COVID-19.

increase with the proportion of the time spent. The amount of ventilation, number of people present, the time spent in an indoor facility, and activities that affect airflow will influence the viral transmission pathways [25].

As there is a minimum virus titre required to cause COVID-19 infection, the viral load emitted in droplets; viability of the virus indoors and outdoors; mechanisms of transmission; concentrations in aerosols; and spatial patterns are important factors. There are uncertainties about SARS-CoV-2 transmission. The relative risk of transmission in various community settings is still unclear, as is the impact of mitigation measures in these contexts. Further, the effect of seasonality and heterogeneities in the population and immunity following the infection is not clear. The masks can protect uninfected individuals from SARS-CoV-2 aerosols and droplets. It is particularly important to wear masks in locations which are likely to accumulate high concentrations of the virus, such as health care settings, airplanes, restaurants, and other crowded places with reduced ventilation. The universal masking and social distancing are two most important sustainable options.

During this unprecedented pandemic outbreak, we have to take special care of the elderly patients. In a geriatric setting, we believe it may be important to stratify patient risk with the Comprehensive Geriatric Assessment (CGA), a multidimensional scale evaluating cognitive, functional, nutritional and welfare aspects. Some of these patients may be frailer than other, such as the older patients with cancer. These patients present with an exponential contagion risk [26]. There are unparalleled therapeutic limitations with COVID-19, as well. Research efforts for treatment options for COVID-19 brought nothing dramatic so far and we have looked in desperation at old treatment option as a saviour including convalescent plasma therapy and repurposing drugs approach [27].

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Chapter 2: Exploring COVID-19: Relating the Spike Protein to Infectivity, Pathogenicity, and Immunogenicity

Background

Introduction-SARS-CoV-2 life cycle: The disease which reportedly began in Chinese city Wuhan in November-December 2019 manifesting as severe respiratory illness, soon spread to various parts of the world, and was named COVID-19, and declared a pandemic by WHO. The life cycle of SARS-CoV-2 begins with membrane fusion mediated by Spike (S) protein binding to the ACE2 receptors. Following viral entry and release of genome into the host cell cytoplasm there occurs replication and transcription to generate viral structural and non-structural proteins. Finally, VLPs are produced and the mature virions are released from the host cell.

Immunogenicity of the spike protein: The S protein is considered the main antigenic component among structural proteins of SARS-CoV-2 and responsible for inducing the host immune response. The neutralising antibodies (nAbs) targeting the S protein are produced and may confer a protective immunity against the viral infection. Further, the role of the S protein in infectivity also makes it an important tool for diagnostic antigen-based testing and vaccine development. The S-specific antibodies, memory B and circulating TFH cells are consistently elicited following SARS-CoV-2 infection, and COVID-19 vaccine shots in clinical trials.

The emerging SARS-CoV-2 variants: The early genomic variations in SARS-CoV-2 have gone almost unnoticed having lacked an impact on disease transmission or its clinical course. Some of the recently discovered mutations, however, have impact on transmissibility, infectivity, or immune response. One such mutation is the D614G variant, which has increased in prevalence to currently become the dominant variant world-over. Another, relatively new variant, named VUI-202012/01 or B.1.1.7 has acquired 17 genomic alterations and carries the risk of enhanced infectivity. Further, its potential impact on vaccine efficacy is a worrisome issue.

Conclusion: The unmet challenges: COVID-19 as a disease and SARS-CoV-2 as its causative organism, continue to remain an enigma. While we continue to explore the agent factors, disease transmission dynamics, pathogenesis and clinical spectrum of the disease, and therapeutic modalities, the grievous nature of the disease has led to emergency authorizations for COVID-19 vaccines in various countries. Further, the virus may continue to persist and afflict for years to come, as future course of the disease is linked to certain unknown factors like effects of seasonality on virus transmission and unpredictable nature of immune response to the disease.

Introduction

The disease which reportedly began in Chinese city Wuhan in November-December 2019 manifesting as severe respiratory illness, soon spread to various parts of the world, and was named COVID-19, and declared a pandemic disease by the World Health Organization (WHO). The disease further spread to certain Arabian and European countries. While these countries were recovering from the epidemic, the disease took hold in the UK and USA, and the South American, Arabian, and South Asian countries, predominantly affecting Brazil, Peru, Iran, and India. Presently, most of the European countries are witnessing recurrent outbreaks and a resurgence of COVID-19, whereas the epidemic is not yet over in other countries [1]. This calls for a need for exploring further the factors affecting the disease transmission, pathogenicity, and immunogenicity, apart from rigorous preventive and control measures, and vigorous vigilance and surveillance.

The life cycle of SARS-CoV-2

The life cycle of SARS-CoV-2 begins with membrane fusion mediated by conformational changes in the Spike (S) glycoprotein triggered by ACE2 receptor binding. The cleavage at the S1/S2 site yields a surface subunit S1, which attaches the virus to the host cell ACE2 receptor, and a transmembrane subunit S2, which mediates the fusion of viral and host cell membranes to facilitate viral entry. Following entry, SARS-CoV-2 releases its genomic RNA into the host cell cytoplasm.

Genome RNA is first translated into viral replicase polyproteins (pp1a and 1ab), which are further cleaved by viral proteases into some 16 non-structural proteins. A replication-transcription complex (RTC) is formed based on the non-structural proteins. In the process of genome replication and transcription mediated by RTC, the negative−sense genomic RNA is synthesized and used as a template to produce positive-sense genomic RNA and sub-genomic RNAs.

The nucleocapsid (N) structural protein and viral RNA are replicated, transcribed, and synthesized in the cytoplasm, whereas other viral structural proteins, including the S protein, membrane (M) protein and envelope (E) protein, are transcribed and then translated in the rough endoplasmic reticulum (RER) and transported to the Golgi complex.

In the RER and Golgi complex, the SARS-CoV-2 glycoprotein is subjected to co-translational and post-translational processing, including signal peptide removal, trimerization, extensive glycosylation and subunit cleavage. The N protein is subsequently associated with the positive sense genomic RNA to become a nucleoprotein complex (nucleocapsid). While S, M, and E proteins as well as other viral proteins are further assembled, followed by budding into the lumen of the ER-Golgi intermediate compartment (ERGIC) to form mature virions.

Finally, the mature virions are released from the host cell.

Exploring SARS-CoV-2 s protein

The virus and spike (s) protein: The novel coronavirus, SARS-CoV-2 is a single-stranded RNA-enveloped virus. Its RNA-based genome is 29,881 bp in length and encodes 9860 amino acids. The SARS-CoV-2 S protein, primarily facilitating the viral entry into the host cells, is a 1273 amino acid homo-trimeric class I fusion protein. The third open reading frame (ORF) in the SARS-CoV-2 genome encodes the S protein, which is the largest protein in the group of four structural proteins including M, E and N proteins [2]. The SARS-CoV-2 S protein bears 76% amino acid sequence identity with SARS-CoV and 93% and 97% amino acid identity with that of the Bat-CoV RaTG13 and Pangolin-CoV, respectively [3]. Due to the conserved residues and binding to the receptors, immunity to SARS-CoV appears to confer a limited immunity to SARS-CoV-2 [4].

The SARS-CoV-2 S protein identifies a transmembrane protein, neuropilin-1 on the surface of human cells, through which it facilitates the virus to attach, invade and infect the host cells [5]. The neuropilins are adhesion molecules responsible for cellular activities such as cell adhesion, survival, repulsion, and attraction.

The structure of s protein: The S protein has a size of 180–200 kDa and length of 1273 aa. The S protein trimers visually form a characteristic bulbous, crown-like protrusions surrounding the viral particle. The S protein is composed of various functional regions or domains. The globular head, S1 subunit contains the N-terminal domain (NTD) and receptor-binding domain (RBD), whereas the stalk, S2 subunit contains the C-terminal (CT) membrane fusion domain followed by the two heptad regions (HR1 and HR2), the transmembrane domain (TMD) and the cytosolic tail (CT). The S protein ectodomain is heavily glycosylated with heterogeneous N-linked glycans and exists in a prefusion and a post-fusion conformation. The associated oligosaccharides influence priming by host proteases and conceal it from antibody recognition [6].

The RBD binds to the aminopeptidase N (APN) region of ACE2 receptors in the host cells [7]. APN is transmembrane glycoprotein expressed on the apical membranes of epithelial cells in the respiratory and enteric tracts, endothelial cells, and renal cells; at synaptic junctions in neural tissues; and on cells of the immune system. While the S1 RBD domain is a highly mutable region, the HR region of the S2 subunit is conserved among HCoVs. The mutations of key residues in RBD can enhance the interactions with ACE2 [8]. The S1 subunit binds with ACE2 to promote the formation of endosomes and triggers the viral fusion activity. The S2 subunit, composed successively of a FP, HR1, HR2, TM domain, and cytoplasmic tail domain (CT), is responsible for viral fusion and entry. The HR1 and HR2 are composed of a repetitive heptapeptide: HPPHCPC, where H is a hydrophobic or traditionally bulky residue, P is a polar or hydrophilic residue, and C is a charged residue (Figure 1).

The S protein exists in a metastable conformation and when the virus interacts with the host cell, extensive structural rearrangement of its components occurs, allowing the virus to fuse with the host cell membrane. The switch in conformations is triggered by RBD-ACE2 receptor binding, which exposes various regions within the S2 subunit [9].

Ace2 receptor binding and viral fusion

To initiate cellular entry, while engaging with ACE2 receptor, the RBD moves like a hinge between two conformations ('up' or 'down') to expose or hide the residues that bind the APN region. Within the RBD, there is a receptor binding motif (RBM) which makes the primary contact with the carboxypeptidase domain of ACE2. SARS-CoV-2 binds to ACE2 with higher affinity than SARS-CoV owing to the presence of a 4-residue motif in the RBM that makes a better contact

with ACE2 than the SARS-CoV S protein. ACE2 as receptors, however, have differential tissue tropism and the structural variations in ACE2 may influence its binding with the S protein [10].

There occurs change in S2 conformation with the RBD bonding with ACE2, which exposes the cleavage sites to cellular proteases, followed by cleavage of the S protein by transmembrane protease serine 2 (TMPRSS2) and other cellular proteases. The cleavage initiates the insertion of FP into the host cell membrane, exposure of the pre-hairpin coiled-coil of the HR1 domain and its interaction with the HR2 to form the six-helical bundle (6-HB). HR1 forms a homo-trimeric assembly in which three highly conserved hydrophobic grooves on the surface binding to HR2 are exposed and bring the viral envelope and cell membrane into proximity for fusion and entry [11].

Whereas other CoVs spike proteins are cleaved at the junction between S1 and S2, the SARS-CoV2 has an additional distinct protease (furin) cleavage site (S2'). With the binding of S1 to the receptor, S2 undergoes an additional cleavage by host proteases through exposure of a second cleavage site (S2′). In the two-step process, the cleavage at the S2′ site activates the protein for membrane fusion and allows fusion of the viral membrane with the host cell membrane. Apart from TMPRSS2, the SARS-CoV-2 S protein can be proteolytically activated by a variety of other cellular proteases including cathepsin B and L (endosomal cysteine proteases), furin, elastase, factor X and trypsin. The protease induced priming and proteolysis initiate the process of cellular entry.

The second cleavage site (S2') changes the conformation of the S protein from the prefusion to the post-fusion state and expands the versatility of SARS-CoV-2 for cleavage by cellular proteases, the tropism and transmissibility owing to the wide cellular expression of furin proteases, especially in the respiratory tract [12]. This also allows the newly synthesised virions can be secreted in a 'preactivated' state ready to fuse with and infect other cells by releasing the FP to disrupt the host cell bilayer-lipid membrane lipid bilayers to prime for fusion without the need to bind to a cellular receptor, such as ACE2. The SARS-CoV-2 S protein has thus evolved further to exploit respiratory cell receptors and proteases to enable enhanced infectivity and rapid spread.

The steps in viral invasion and internalisation, thus, include binding of the virus to the cell surface, alteration of the conformation of the S protein, proteolysis of the S protein, and release of the S2 subunit followed by fusion of the virion and endocytosis. The S protein binds to the host cell by recognizing the receptor ACE2, which is a homolog of ACE and distributed in the lungs, intestines, heart and vasculature, and kidneys. The alveolar epithelial type II cells are the major expressing cells. SARS-CoV-2 S binds to human ACE2 with a dissociation constant (KD) of 14.7 nM, which for SARS-CoV S is 325.8 nM, indicating that SARS-CoV-2 S is more sensitive to ACE2 than is SARS-CoV S [13].

The S subunits exist in a noncovalent form in the uncleaved state and cleavage of S1 and S2 subunits by host proteases, initiates the fusion process. The proteases furin and neuropilin-1 (NRP1), which are abundantly expressed in the respiratory and olfactory epithelium, with highest expression in endothelial and epithelial cells, bind the substrates and potentiate the SARS-CoV-2 infectivity [14]. Trypsin is another host cell protease that can cleave the S protein. Viral fusion process refers to fusion of the viral membrane and host cell membrane, followed by internalisation and the release of the viral genome into the host cell.

Immunogenicity of the s protein

The experimental immunological studies: The S protein is considered the main antigenic component among structural proteins of SARS-CoV-2 and responsible for inducing the host immune response. The neutralising antibodies (nAbs) targeting the S protein are produced and may confer a protective immunity against the viral infection. The RBD region is a critical target for nAbs for preventive as well as blocking therapy with antibodies. Further, the role of the S protein in infectivity also makes it an important tool for diagnostic antigen-based testing and vaccine development [15]. On the other hand, several monoclonal antibodies (mAbs) have shown promising results in neutralizing SARS-CoV-2. CR3022, a SARS-CoV-specific human mAb, binds potently with SARS-CoV-2, and has potential as therapeutic as well as prophylactic agent alone or in combination with other nAbs, for SARS-CoV-2 infection. In addition, sera from SARS patients during convalescence or animals specifically immunized with SARS-CoV S1 may cross-neutralize SARS-CoV-2 and reduce viral load or S protein-mediated SARS-CoV-2 entry.

Yuan et al have determined the structure of CR3022, the neutralizing antibody obtained from convalescent COVID-19 patients in complex with the receptor-binding domain of SARS-CoV-2 spike [16]. While Yu, et al. designed a series of prototype DNA vaccines against the SARS-CoV-2 S protein [17]. The analysis of the vaccine candidates in rhesus macaques

have shown that the animals developed protective humoral as well as cellular immune responses when challenged with the virus. Neutralizing antibody titers were also observed at levels similar to those seen in humans who have recovered from SARS-CoV-2 infection [18].

In addition, the peptide fusion inhibitors such as nelfinavir mesylate, suppress both SARS-CoV-2 S and SARS-CoV S-mediated the virion-cell fusion. Nelfinavir may also inhibit TMPRSS2 involved in activation of the S protein. Camostat mesylate, a protease inhibitor targeting SARS-CoV-2 S cleavage sites, is a potent serine protease inhibitor of TMPRSS2 and appear to block the SARS-CoV-2 cellular entry [19].

The COVID-19 pandemic has dramatically accelerated global vaccine development efforts, most targeting the viral S protein. There are SARS-CoV-2 vaccine candidates based on types DNA- and RNA-based formulations, recombinant subunits containing viral epitopes, adenovirus-based vectors, and inactivated virus, under development. The inactivated virus vaccines have been traditionally used. Thus, inactivated virus vaccine development for SAR-CoV-2 may be a time-tested and effective way for the prevention of disease [20]. Gao, et al. developed experimentally a purified inactivated SARS-CoV-2 virus vaccine candidate, PiCoVacc, which was able to induce SARS-CoV-2–specific neutralizing antibodies in mice, rats, and nonhuman primates. These antibodies neutralized various SARS-CoV-2 strains, thus displaying a possible broader neutralizing ability. The animals were immunized with one of two vaccine doses and then inoculated with SARS-CoV-2. Those having received the lowest dose showed signs of controlling the infection, whereas those receiving the higher dose appeared more protected with undetectable viral load in the pharynx or lungs at 7 days after infection.

The immune response to S protein: In the early phase of SARS-CoV-2 infection, T- and B-cell counts are substantially decreased [21]. In a period of about 2 weeks following onset of symptoms, IgM and IgG become detectable. The T cells play an important role in supporting the development of the B cell response, while the B cells produce the antibodies that recognize SARS-CoV-2 S protein. A particular subset of T cells, called T-follicular helper (TFH) cells, is predictor of an effective immune response. The circulating TFH cells are S-specific and functional, and the occurrence of CXCR3+ TFH cells is positively correlated with neutralising antibody titres in COVID-19-convalescent individuals [22].

The S glycoprotein plays essential roles in virus attachment, fusion, and entry into the host cell. Simultaneously, its surface location renders it a directly accessible target for host immune response [23]. The SARS-CoV-2 RBD has a high hACE2 binding affinity than SARS-CoV to support efficient cell entry. Further, SARS-CoV-2 RBD is less exposed than SARS-CoV RBD and helps the virus to evade immune response. Furthermore, the SARS-CoV-2 spike contains a proprotein convertase (PPC) motif at the S1/S2 boundary and its prior cleavage during viral packaging enhances the efficiency for entry into new target cells including those with low expression of TMPRSS2 and other proteases [24].

The immune system responds to the S protein, both S1 and S2 subunits being highly antigenic [25]. As a result, the S-specific antibodies, memory B and circulating TFH cells are consistently elicited following SARS-CoV-2 infection and COVID-19 vaccine shots in the clinical trials (Figure 2).

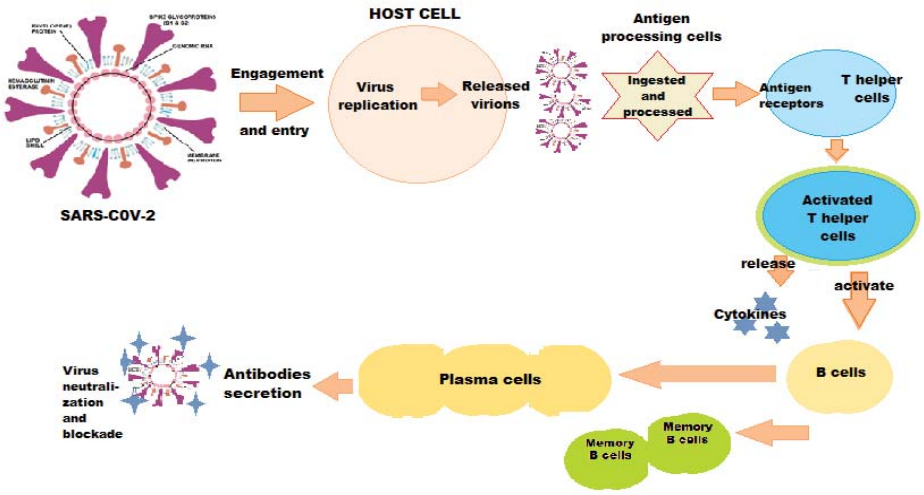

Figure 2: Immune Response against SARS-CoV-2.

There evolves humoral and circulating TFH immunity against S protein in the convalescent COVID-19 patients and those with robust B cell response show a strong plasma neutralising activity [26]. Still, in the COVID-19-convalescent individuals, the titres of spike-specific neutralising antibodies are variable [27]. In the clinical studies, the convalescent individuals who experienced severe COVID-19 showed higher neutralising antibody titres, a faster increase in lymphocyte counts and a higher frequency of CXCR3+ T follicular help (TFH) cells compared with COVID-19-convalescent individuals who experienced non-severe disease [28]. Remarkably, those recovering from severe COVID-19 elicit and maintain higher antibody and neutralization titres than the non-severe group. Thus, the neutralising antibody titre appears to correlate with the severity of the disease [29].

These findings provide insights into neutralising antibody responses in COVID-19-convalescent individuals and may facilitate the treatment and vaccine development for SARS-CoV-2 infection. The neutralising antibodies are crucial in protecting from re-infection. They bind to the S protein as well as prevent it from getting to attached to the host cells to facilitate virus entry [30]. The presence of anti-spike or anti-nucleocapsid IgG antibodies has been associated with a substantially reduced risk of SARS-CoV-2 reinfection in the ensuing 6 months [31]. Thus, generating a strong neutralising antibody response is the primary goal for SARS-CoV-2 vaccines.

Associated immunological phenomena: *Antibodies predating COVID-19 infection:* It has been documented that immunological memory after infection with seasonal human coronaviruses (hCoVs) may potentially contribute to cross-protection against severe acute SARS-CoV-2 infection [32]. It has been seen that a proportion of SARS-CoV-2–uninfected persons have circulating immunoglobulin G (IgG) antibodies that could cross-react with the S2 subunit of the SARS-CoV-2 S protein. By contrast, the COVID-19 patients generate IgA, IgG, and IgM antibodies that recognise both the S1 and S2 subunits.

Experimentally, these anti-S2 antibodies from SARS-CoV-2–uninfected patients have neutralising activity against both SARS-CoV-2 and SARS-CoV-2 S pseudo-types. A much higher percentage of SARS-CoV-2–uninfected children and adolescents show these antibodies compared with adults, which may be because children and adolescents generally have higher hCoV infection rates and a more diverse antibody repertoire, which may explain the age distribution of COVID-19 susceptibility [33].

Similarly, the study by Demers-Mathieu et al. has evaluated the presence and the levels of antibodies reactive to SARS-CoV-2 S1 and S2 subunits in the human milk. These antibodies are reactive to S protein and nucleocapsid protein and could provide passive immunity to breastfed infants and protect from COVID-19 [34].

There exists striking structural similarity and sequence conservation between the SARS-CoV-2 S and SARS-CoV S glycoproteins that recognize hACE2 to enter the host cells. This resemblance is further strengthened by finding that SARS-CoV S elicits polyclonal antibody (pAb) response which can potently neutralise the SARS-CoV-2 S-mediated entry into the cells. Most of the SARS-CoV neutralising Abs target the SB domain and several of them recognize the RBM and prevent receptor engagement [35].

Antibody dependent enhancement: The SARS-CoV-2 inactivated whole-virion vaccine candidates carry the risk of viral infection of Fc receptor (FcR)-expressing cells, a phenomenon called antibody-dependent enhancement (ADE). Similarly, all other SARS-CoV-2 vaccine candidates entering clinical trials contain or express full-length or near full-length S protein and therefore also bear risk of ADE, albeit less so [36]. Considering this phenomenon, the continued search for a safe and effective SARS-CoV-2 vaccine may appear a farther dream but should go on.

The recombinant RBD proteins of SARS-CoV and MERS-CoV have been shown to potently induce protective nAbs. Further, it has been shown that the RBD part within RBD-Fc fusion protein is responsible for induction of nAbs against SARS-CoV-2. Furthermore, the anti-SARS2-RBD sera have been found to cross-react with SARS-RBD. Therefore, the observed cross-binding with SARS-RBD and cross-neutralization of SARS-CoV is likely to be contributed by antibodies targeting the conserved SARS-CoV-2 core subdomain, which contains cross-neutralization antibody epitopes. Zang, et al. have documented that the anti-RBD sera exhibited potent neutralization effects on SARS-CoV-2. Moreover, the anti-RBD sera also inhibited SARS2-S-mediated cell–cell fusion. On this basis, it seems that the anti-RBD antibodies do not promote ADE [37].

Development of COVID-19 vaccines: Various SARS-CoV-2 vaccine candidates based on various vaccine platforms, such as inactivated or live attenuated vaccines, DNA and mRNA vaccines, viral vector-based vaccines, and recombinant

protein-based vaccines, have been developed [38]. Most of these vaccine strategies are based on the full-length S glycoprotein, which is the major SARS-CoV-2 surface antigen. Though the S protein is thought to be a promising vaccine immunogen for generating protective immunity, optimizing antigen design is critical to ensure an optimal immune response through exposing more neutralising epitopes and displaying fewer potentially weakly or non-neutralising epitopes.

There is fear of erratic immune response to the S protein-based vaccines [39]. The RBM appears the most immunodominant neutralising epitope of the whole S protein, capable of readily eliciting strong neutralising antibody response but presents difficulties in designing and developing vaccine because of certain issues as follows -

The native trimeric SARS-CoV-2 S protein conceals its immunodominant RBMs by adopting the closed conformation. The SARS-CoV-2 evades immune surveillance also through conformational masking [40].

The S1 subunit spontaneously dissociates from the S glycoprotein as a trimer to assume the RBD closed conformation, leaving only the post-fusion S2 trimer. The resulting S1 and S2 subunits might expose immunodominant, non-neutralising epitopes that are utilized by SARS-CoV-2 to serve as decoys to distract the host immune system, inducing a large proportion of ineffective antibody responses.

Further, the vaccine candidates based on the full-length S protein of the closely related SARS-CoV could elicit neutralising antibody responses against infection of SARS-CoV, which may also induce harmful immune responses, including liver damage of the vaccinated person, infection of human immune cells by SARS-CoV, and antibody-dependent enhancement of SARS-CoV infection.

Besides the RBD, which has been shown to be a major target for human neutralising antibody responses, the NTD has recently been identified to be a new vulnerable site of the SARS-CoV-2 S protein for antibody mediated neutralization and therefore could also serve as a recombinant protein-based vaccine [41]. The NTD-specific neutralising antibodies are likely to target the S protein in both closed and open conformations. In addition, the apparent accessibility of the FP and HR1 region in the SARS-CoV-2 S ectodomain trimer may be good immunogen candidates for epitope-focused vaccine design aimed at raising effective CoV neutralising antibodies.

Emerging SARS-CoV-2 Strains

Mutations and variation in SARS-CoV-2: Understanding the nucleotide variations in the SARS-CoV-2 genome provides a useful insight for the evolution of the disease and the propagation of the pandemic [42]. The early variations in SARS-CoV-2 have made their way almost unnoticed as the virus spread around the world. Whereas most variations or mutations have no impact on the viral ability to transmit or cause disease, some mutations appear to have impact on transmissibility, infectivity, or lethality. Some of these mutations have possibly arisen because of the virus evolving from immune selection pressure in infected individuals and are more prevalent in patients with mild than those with severe disease. In general, the mutations can be interpreted as a strategy through natural selection to facilitate extensive spread of the viral infection.

Though, the SARS-CoV-2 virus has a low mutation rate by virtue of the nsp14 protein acting as a 3′-5′ exoribonuclease on both single-stranded and double-stranded RNA during the viral replication cycle [43]. Still its large genome appears to facilitate recombination, insertions, and deletions. Andrés et al found that the viral S protein accumulates deletions upstream and close to the S1/S2 cleavage site [44]. Further, SARS-CoV-2 can resort to RNA viral evolution through recombination (synthesis of chimeric RNA molecules from two different progeny genomes) and reassortment (the packaging within a single virion of genomic segments from different progeny viruses.

The single nucleotide variations (SNVs) as SARS-CoV-2 Spike amino acid replacements in the receptor binding domain (RBD) occur relatively frequently [45]. There is recurrent emergence and significant onward transmission of a six-nucleotide deletion in the Spike gene resulting in loss of two amino acids labelled as ΔH69/ΔV70. This deletion often co-occurs with the receptor binding motif amino acid replacements N501Y, N439K and Y453F. As such, these deletions have been found in a small percentage (2.2%) of the samples [46]. Currently the major SARS-CoV-2 Lineages are A, B, B.1, B.1.1, B.1.177. The lineage - B.1.1.7, of present concern, was first sequenced on 20 Sep 2020 and is spreading from the UK and has been discovered in Denmark, the Netherlands, Italy, Israel, Australia, Hong Kong, and Singapore. Other countries are being increasingly involved. Taking a note of the major mutations, their lineages and effects on disease transmissibility is important to understand the changing face of the pandemic (Table 1).

Mutation	Lineage	Effects
D614G	B.1	Moderate effect on transmissibility
A222V	B.1.177	Fast growing lineage. No mutation effects documented
N439K	B.1.141 B.1.258	Eludes some mAbs Enhanced affinity for hACE2 receptor binding
Δ69-70	B.1.1 B.1.258	Evades immune response Diagnostic failure in assays targeting S gene
N501Y	B.1.1.7	Fast growing lineage Enhanced affinity for hACE2 receptor binding
N501Y + Δ69-70	B.1.1.7	Fast growing lineage Evades immune response Enhanced affinity for hACE2 receptor binding
N439K + Δ69-70	B.1.256	Evades immune response Enhanced affinity for hACE2 receptor binding
Y453F	B.1.1 B.1.1.298	Eludes some mAbs Enhanced affinity for hACE2 receptor binding

Table 1: Major mutations, lineages and effects on disease transmissibility and course.

Using the complete sequences of 1,932 SARS-CoV-2 genomes, six types of the strains have been identified. The 13 signature variations in the form of SNVs in protein coding regions and one SNV in the 5′ untranslated region (UTR) provide interpretation for the six types (types I to VI). The type VI, characterized by the four signature SNVs C241T (5′UTR), C3037T (nsp3 F924F), C14408T (nsp12 P4715L), and A23403G (Spike D614G), with strong allelic associations, first reported in China, has become the dominant type world over. Out of these, C241T is in the 5′ UTR appears to be of uncertain significance. The other three SNVs, 3037T-14408T-23403G characterising the increasing frequency of the type VI, in majority of samples from various regions suggests a possible fitness gain for the virus. Further, the strains missing one or two of these signature SNVs fail to persist or wiped out by the evolutionary more fit variants [47].

Emergence of the D614G variant: The genome analysis of various isolates of the SARS-CoV-2 shows several regions having an increased proportion of some variants. One such variant is the D614G mutation in the C-terminal end of the S1 domain and in proximity to the S2 subunit. This variant has increased in prevalence with rapidity, becoming the predominant variant now world-over [48]. The D614G variant is associated with the faster viral transmission and harbouring and discharge of higher viral loads [49]. The variant has been found to be associated with increased Infectivity and reduced S1 Shedding [50]. Thus, the presence of glycine at 614 appears to improve the S-Protein Stability and Increase its incorporation into virions. Further, the structural analyses have revealed that the RBD of the D614G form of the spike protein is more likely to assume an “open” conformation than the RBD of the ancestral D614S form, implying an improved ability to bind to the hACE2 receptor. Furthermore, the higher viral load with D614G is consistent with epidemiological data suggesting enhanced infectivity associated with D614G [50].

The studies in hamsters infected with D614S or D614G variants, Plante et al. have documented that the contemporary D614G variant replicated to higher titers in nasal-wash samples early after infection and outcompeted the ancestral D614S variant. These findings suggest increased viral fitness for D614G in a major upper airway compartment potentially associated with enhanced transmission [51]. The D614G variant was found to be equally sensitive to neutralizing antibodies and did not cause more severe disease than the ancestral strain in hamsters, a finding that supports current findings in humans [52].

Emergence of VUI-202012/01 variant: Another new variant, named VUI-202012/01 (Variant under Investigation, year 2020, month 12, variant 01), has been identified through viral genomic sequencing in the United Kingdom (UK). It has 17 mutations that may lead to a conformational change in the shape of the virus including the S protein. There is an N501Y mutation in the S protein, related to part alteration in the receptor-binding domain (RBD) and may result in the virus becoming more infectious. There are multiple spike protein mutations such as a double deletion (positions 69 and 70), deletion 144, N501Y, A570D, D614G, Y453F, P681H, T716I, S982A, D1118H) as well as mutations in other genomic regions. The preliminary analyses in the UK suggests that the UK variant B.1.1.7 has multiple mutations on it

and is significantly more transmissible than previously circulating variants, with an estimated potential to increase the reproductive number (R) by 0.4 or greater with an estimated increased transmissibility of up to 70percent [53].

The variant was first detected during October 2020 in the UK from a sample taken in Sep 2020. The backwards tracing using genetic evidence suggests this new variant first emerged in September 2020 and then circulated at low levels in the population until mid-November, and thereafter it has spread alarmingly by mid-December [54]. It has been correlated with a significant increase in the rate of COVID-19 infection in the UK. The cases with the new variant have been from Denmark, Netherlands, and Belgium. The VOC 202012/01 variant has not been identified so far in the United States [55].

The B.1.1.7 variant has acquired 17 changes - 14 non-synonymous (amino acid [AA] altering) mutations, 6 synonymous (non-AA altering), and 3 deletions. The deletions include 69/70 deletion and P681H - near the S1/S2 furin cleavage site, in the S protein, and ORF8 stop codon (Q27stop) - in open reading frame 8. As reported by the UK, there is no clear epidemiological link to animals for VUI 202012/01 [56].

- The emergence of VUI-202012/01 has certain likely implicatThe probability of a wider spread of the new virus variant across the European nations and continents
- The potential impact on SARS-CoV-2 diagnostics - The UK reports that the deletion 69-70 in the spike protein of the variant causes a negative result from S-gene RT-PCR assays applied in some laboratories in the UK. This specific mutation has occurred many times in different countries and is geographically widespread.
- The potential impact on severity of disease in a population or groups - Potential impact on severity of disease in a population or group - The available information regarding severity of the new virus variant is limited. To date, there is no indication of increased infection severity observed related to the variant, but the assessment is challenged by the fact that the majority of cases were reported in people under 60 years old, who are less likely to develop severe symptoms.
- Rapid increase of a SARS-CoV-2 variant with multiple spike protein mutations observed in the UK. None of the previously described SARS-CoV-2 variants have been shown to cause increased infection severity; on the contrary, a clade 19B variant with lower severity was detected in Singapore in the spring and then disappeared.
- Frequency of reinfections - The mutations observed in the new variant are related to the receptor binding site and other surface structures, which may alter the antigenic properties of the virus. Based on the number and location of spike protein mutations, it seems likely that some reduction in neutralisation by antibodies will be seen, but there is as yet no evidence that there is a resulting impact on increased risk for reinfection or lower vaccine effectiveness.
- The potential impact on vaccine effectiveness is a worrisome issue. It is being conjectured that the efficacy COVID-19 vaccines will remain like the ancestral SSARS-CoV-2 virus.

Recently a distinct phylogenetic cluster (named lineage B.1.1.7) was detected within the COG-UK surveillance dataset. This cluster has been growing rapidly over the past 4 weeks and since been observed in other UK locations, indicating further spread [57]. The B.1.1.7 lineage accounts for an increasing proportion of cases in parts of England. The number of B.1.1.7 cases, and the number of regions reporting B.1.1.7 infections, are growing. B.1.1.7 has an unusually large number of genetic changes, particularly in the spike protein. Three of these mutations - mutation N501Y in the receptor-binding domain (RBD) leads to increased binding affinity to human ACE2; the spike deletion 69-70del has been associated with evasion to immune response; and mutation P681H is immediately adjacent to the furin cleavage site.

Other isolated mutations: P681H Mutation: The P681H is S protein mutation and involves the S1/S2 furin cleavage site. It has also emerged spontaneously multiple times and has been recently reported from Nigeria. There is no evidence to indicate that the P681H variant is contributing to increased transmission of the virus in Nigeria [58]. Earlier the analysis of samples collected in Aug 2020 and sequenced at the African Centre of Excellence for Genomics of Infectious Diseases (ACEGID), Nigeria, identified two SARS-CoV-2 sequences that share one non-synonymous SNP in S protein in common with this lineage. The non-synonymous SNV, S:P681H, has been observed in global data outside of the B.1.1.7 lineage.

At the moment, only about 1% of the SARS-CoV-2 genomes from Nigeria share any of the 17 protein-altering variants from the UK lineage of concern (B.1.1.7). Other reported mutations such as the N501Y, A570D, and the HV 69 - 70 deletion in S protein have not been detected in Nigeria currently.

ORF8 stop codon (Q27stop): This mutation is not in the spike protein but involves the open reading frame 8. Similar mutations have occurred in the past. In Singapore, one strain with this type of mutation emerged and disappeared. This is in line with the understanding that SARS-CoV-2 ORF8 is poorly conserved among coronaviruses. Among accessory genes, open reading frame 8 (ORF8) stands out by being highly variable and may be related with the virus ability to spread [50].

Conclusion: The pandemic and unmet challenges

COVID-19 as a disease and SARS-CoV-2 as its causative organism, continue to remain an enigma. While we continue to explore the agent factors, disease transmission dynamics, pathogenesis and clinical spectrum of the disease, and therapeutic modalities, the grievous nature of the disease has led to emergency and non-emergency authorizations for COVID-19 vaccines in various countries around the world.

Challenges posed by virus mutants

The genome data outlines that two SARS-CoV-2 virus samples collected from anywhere in the world differ by an average of just 10 RNA letters out of 29,903 [60]. Thus, there is a sluggish mutation rate. Despite this, through extensive research, researchers have catalogued more than 12,000 mutations in SARS-CoV-2 genomes. Many mutations appear not to affect the virus's ability to spread or cause disease. In fact, the mutations altering the viral structural proteins are more likely to harm a virus and make it extinct rather than improve it. Further, various SARS-CoV-2 strains have no major impact but might in future on the course of the disease and pandemic, as they accumulate. Even an insignificant mutation can have a profound by downgrading the immune response.

The D614G mutation leads to favour open conformations and the viral entry into the host cells. Further, this mutation It is often accompanied by three mutations in other parts of the SARS-CoV-2 genome. The variation increases density of the S protein and viral infectivity [61]. There has been a rapid spread of D614G which was first spotted in samples collected early in the course of the pandemic from China and Germany. It has become the dominant strain across the European continent, Australia, Canada, and parts of the United States, and predicted to involve rest of the world regions. It appears that D614G represents a more transmissible form of SARS-CoV-2, which has emerged as a product of natural selection [62]. There is fear that a similar situation may occur with the VUI-202012/01 or B.1.1.7 variant.

Ill-defined and short-lived immune response

The studies in mice, monkeys and humans that received one of the experimental RNA vaccines, produced antibodies that proved more potent at blocking G viruses than D viruses [63]. With the G strain now ubiquitous, these findings are encouraging. Though, the experience with HIV that mutates to elude various vaccines developed so far, makes the researchers wary of the potential of SARS-CoV-2 to evade immunity and antigenic responses. The historic trial with the common cold Corona vaccine in the late 1980s, should be mentioned in this context, which included 15 healthy people given nasal solution containing inactivated common cold coronavirus. The monitoring for one year followed by analyses documented that most of them became infected before developing an effective immune defence. So far, various SARS-CoV-2 strains do not stop the immune system from developing neutralising antibodies that recognise the virus. Still, there is a possibility that the virus may acquire mutations that either evade the immune response or alter the susceptibility to antibodies and immune cells.

In the experimental studies using animals and cell cultures, along with the latest molecular techniques, and in small human clinical studies, the immune response to SARS-CoV-2 has been recognised [64]. But it is not certain whether the immunity will be effective or lasting. Whether a vaccine will provide adequate protection, whether those who have recovered from COVID-19 can return to pre-pandemic behaviours and how readily the world can reduce the threat posed by the disease. The reinfections have been shown to occur [65]. Neither the frequency nor the elements of the immune response associated with reinfection have been studied and well documented.

The future course of COVID-19 pandemic

The, virus SARS-CoV-2 is here to stay for the foreseeable future [66]. The future of the disease, of course, appear to depend on various unknown factors, which include the effects of seasonality on the disease transmission and spread, the degree and duration of effective immune response to the disease, and the life-style choices made by individuals and measures enforced by the governments. Further, the pandemic's course will also depend on the availability of a COVID-19

vaccine, it's effectively and duration of its protective immune response. Concerning the latter, the significance of viral mutations should also be borne in mind and highlighted. There are serious challenges posed by SARS-CoV-2 virus and COVID-19 as the disease to the humanity. Let us hope that the today's unmet challenges are solved in near future.

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Chapter 3: Stages in COVID-19 vaccine development: The Nemesis, the Hubris, and the Elpis

Background

The nemesis – SARS-CoV-2 pandemic: Leaving in its wake millions of infections, accompanied by an immense magnitude of morbidity and multitude of mortality, and an unfathomable economic toll, the COVID-19 pandemic has led to a global calamity. An effective and safe COVID-19 vaccine is urgently needed to prevent the disease, thwart the complications and avert deaths resulting from unrestrained transmission of the infection.

THE hubris – Vaccine development: While most of the platforms of vaccine candidates have focused on the spike (S) protein and its variants as the primary antigen of COVID-19 infection, various techniques involved include nucleic acid technologies (RNA and DNA), non-replicating viral vectors, peptides, recombinant proteins, live attenuated and inactivated viruses. There are novel vaccine technologies being developed using next-generation strategies for precision and flexibility for antigen manipulation relating to SARS-CoV-2 infection mechanisms.

The elpis – Updates and prospects: There were nine different technology platforms under research and development to create an effective vaccine against COVID-19. Although there are no licensed vaccines against COVID-19 yet, there are various potential vaccine candidates under development and advanced clinical trials. Out of them, one having undergone phase III clinical trials, has become available in some countries for use among the high-risk groups following emergency use authorization. Other COVID-19 vaccines may soon follow the suit.

Conclusion – Hopes and concerns: The hope of benefiting from the vaccine to the extent that it may be the only way to tide over and control the COVID-19 pandemic, is accompanied by the likely fear of adverse effects and opposition in public for COVID-19 vaccination, including the vaccine hesitancy. Further, there is concern among scientific circles that vaccine may have opposite of the desired effect by causing antibody-dependent disease enhancement.

Introduction

Curbing the infection

Leaving in its wake millions of infections, accompanied by an immense magnitude of mortality and multitude of morbidity, and an unfathomable economic toll, the COVID-19 pandemic has led to a global calamity [1]. The disease has seriously affected the vulnerable groups in the society including those 65 years of age or older, persons with underlying conditions, and the economically deprived populations. It is feared that more of devastation is likely to be witnessed in form of serious post-COVID-19 complications especially in the survivors of the serious illness. An effective and safe vaccine is thus urgently needed to prevent the disease, thwart the complications and avert deaths resulting from unrestrained transmission of the infection [2].

Although there are no licensed vaccines against COVID-19 yet, there are various potential vaccine candidates based on a variety of platforms including lipid nanoparticle mRNA, DNA, adjuvanted protein, inactivated virus particles, and non-replicating viral vectors are in various phases of clinical trials, including 11 vaccine candidates in phase 3 trials, followed by over a hundred vaccine candidates in preclinical testing [3].

The safety and immunogenicity data relating the vaccines in this context are important. The clinical significance of SARS-CoV-2 binding and neutralizing antibody titres and their ability to predict efficacy is needed to be evaluated and confirmed. Though the immune correlates of protection against SARS-CoV-2 are yet to be determined, the neutralising antibodies are thought to be associated with protection based on results from studies in COVID-19 non-human primate challenge models inferring that neutralising antibody response is correlated with protection [4]. These findings have led to the use of neutralisation assays to assess immune responses in recent human COVID-19 vaccine trials [5].

The nemesis: SARS-CoV-2 virus invasion and the pandemic

The virus and the disease: During December 2019, an outbreak of apparently viral pneumonia was reported from the city of Wuhan, in Hubei province, China. Soon the disease spread to other parts of China and several countries to become a pandemic. By 9 January 2020, it was established that the disease was caused by a novel coronavirus, 2019-nCoV or SARS-CoV-2 and was named COVID-19 [6]. Later, during the first half of January 2020, the Chinese researchers shared

the genome sequence of the virus, followed by identification of the same by the Mutualized Platform for Microbiology (P2M), Pasteur Institute, Paris on 29 January 2020 from the samples taken from the initial suspected patients in France [7].

Following the sequencing of SARS-CoV-2 genome, an international response was triggered to develop a prophylactic vaccine to provide acquired immunity against COID-19. By April 2020, over hundred institutes and companies in 19 countries were working on the vaccine for COVID-19. Initially it was said, including by WHO in February 2020, that a vaccine for the disease was not expected to become available in less than 18 months. Later, it has been claimed by researchers that with the help of genetic Engineering, the COVID-19 Vaccine can possibly be made in months rather than years [8].

Various vaccines under development to combat the COVID-19 have been modelled on the original strain, common among hCoV-19 genetic sequences published during the initial months of the course of the disease pandemic. Understanding the evolution and mutations of SARS-CoV-2 during the COVID-19 pandemic is imperative for disease control and prevention through the vaccine programme. A spike protein mutation D614G has emerged through supplanting aspartic acid (D) in the 614th position of the amino acid with glycine (G), hence the change known as D614G.

The D614G mutation has supposedly enhanced viral replication in human airway tissues, enhanced viral survival in the upper airway of infected hamsters, and increased susceptibility to neutralization. It appears that the mutation may have increased the infectivity of the virus [9]. The work by Plante et al underlines the importance of this mutation in viral spread, vaccine efficacy, and antibody therapy [10]. It has been pointed out, the vaccines against COVID-19 are hoped to work against new G-strain, as well [11]. Further, the study involving Hamsters concluded that the D614G mutation may not reduce the ability of vaccines in clinical settings to protect against COVID-19 and the neutralising antibodies are to be assessed against the circulating variant of the virus before clinical development.

SARS-CoV-2 genomic sequencing: The genome of SARS-CoV-2 is comprised of a single-stranded positive-sense RNA. It is composed of 13–15 (12 functional) open reading frames (ORFs) containing ~30,000 nucleotides and contains 38% of the guanine-cytosine (GC) content and 11 protein-coding genes, with 12 expressed proteins [12]. The ORFs are arranged as replicase and protease (1a–1b) and major S, E, M, and N proteins. These gene products play important roles in viral entry, fusion, and survival in host cells.

Basically, the genomic sequencing is a technique to interpret genetic information found within the virus. So far, there are over 1,000 COVID-19 genomes published worldwide [13]. The genomic sequencing helps in understanding when and where the version of the virus originated and how the virus is evolving. Sequencing the genome of SARS-CoV-2 virus also helps in understanding the disease transmission kinetics, its spread in population groups and planning and evaluating the containment efforts. In addition, it helps to track the viral mutations as the disease spreads.

In general, the viruses circulating locally have small genetic changes compared to the ones that are circulating elsewhere. Thus, the genomic sequence can be used to estimate the infected population size and how the virus is spreading. Further, understanding of the genomic structure of the virus helps in developing drugs and vaccines for therapy as well as prophylaxis of COVID-19.

The hubris: COVID-19 vaccine development programmes

The basis for vaccine development: It has been documented that the immunological memory after infection with seasonal human coronaviruses (hCoVs) may potentially contribute to cross-protection against SARS-CoV-2. In a cohort of 350 SARS-CoV-2–uninfected individuals, a small proportion had circulating immunoglobulin G (IgG) antibodies that could cross-react with the S2 subunit of the SARS-CoV-2 spike protein [14]. The anti-S2 antibodies from SARS-CoV-2–uninfected patients show specific neutralizing activity against both SARS-CoV-2 and SARS-CoV-2 S pseudo-types. These antibodies are present in a higher proportion of SARS-CoV-2–uninfected children and adolescents compared with other age groups. By contrast and notably, convalescent COVID-19 patients generate IgA, IgG, and IgM antibodies that recognize both the S1 and S2 subunits.

The fact that viral S protein elicits an antibody response is the cornerstone for development of a vaccine to protect against SARS-CoV-2 infection through generating a strong neutralizing antibody response [15]. In fact, the COVID-19 pandemic has dramatically expedited global vaccine development efforts, most targeting the viral spike (S) glycoprotein.

The S protein, which is localized on the virus surface and engages through the host cell angiotensin-converting enzyme 2 (ACE2) receptors. Eliciting neutralizing antibodies that block S-ACE2 interaction or indirectly prevent membrane fusion, constitute an attractive modality for vaccine-elicited protection. Further, the S protein is crucial in inducing neutralizing antibodies to protect from re-infection. The neutralizing antibodies not only bind to the viral spike protein, but also prevent it from being able to attach to and enter human cells.

The immune response to SARS-CoV-2 spike protein especially in relation to B and T cells has been investigated. Yu, et al. designed a series of prototype DNA vaccines against the SARS-CoV-2 spike protein, which is used by the virus to bind and invade human cells [16]. Analysis of the vaccine candidates in rhesus macaques showed that animals developed protective humoral and cellular immune responses when challenged with the virus. There were observed neutralizing antibody titres at levels similar to those seen in patients recovered from SARS-CoV-2 infection [17]. Most antibodies isolated from the COVID-19 patients are specific to SARS-CoV-2 virus. However, COVA1-16 is a relatively rare antibody that also cross-neutralizes SARS-CoV [18]. Further, Yuan et al have documented the structure of CR3022, a neutralizing antibody obtained from convalescent COVID-19 patients in complex with the receptor-binding domain of SARS-CoV-2 spike [19].

The B cells are responsible for producing the antibodies that recognize SARS-CoV-2, while T cells play an important role in supporting the development of the B cell response. The subjects showing a strong neutralizing antibody activity have a robust B cell response. Further, a particular subset of T cells, called T-follicular helper cells, has been found to be a predictor of an effective immune response. The T-follicular helper cells are involved in helping B cells to make antibodies. The humoral and circulating follicular helper T cell (cTFH) immunity against spike in recovered COVID-19 patients, S-specific antibodies, memory B cells and cTFH are consistently elicited after SARS-CoV-2 infection, indicating robust humoral immunity and positively associated with plasma neutralizing activity, whereas there is comparatively lower frequency of B cells or cTFH specific for the receptor binding domain of S protein [20]. These findings are, thus, consistent with the immune response in patients recovered from COVID-19.

The COVID-19 vaccine platforms: While most of the platforms of vaccine candidates have focused on the spike (S) protein and its variants as the primary antigen of COVID-19 infection, various techniques involved include nucleic acid technologies (RNA and DNA), non-replicating viral vectors, peptides, recombinant proteins, live attenuated viruses, and inactivated viruses [21]. The main protein, S protein to boost the immune system can be given as a vaccine in many different forms such as inactivated (dead) virus, as expressed protein, in a DNA or RNA vector that will lead the cells to make this protein and stimulate to make antibodies and activate T cells to control the viral infection or eliminate the infected cells to reduce disease severity and complications (Figure 1).

As reported during September 2020, there were nine different technology platforms (Table 1) under research and development to create an effective vaccine against COVID-19 [22].

There are novel vaccine technologies being developed for COVID-19 using next-generation strategies for precision and flexibility for antigen manipulation on COVID-19 infection mechanisms [23].

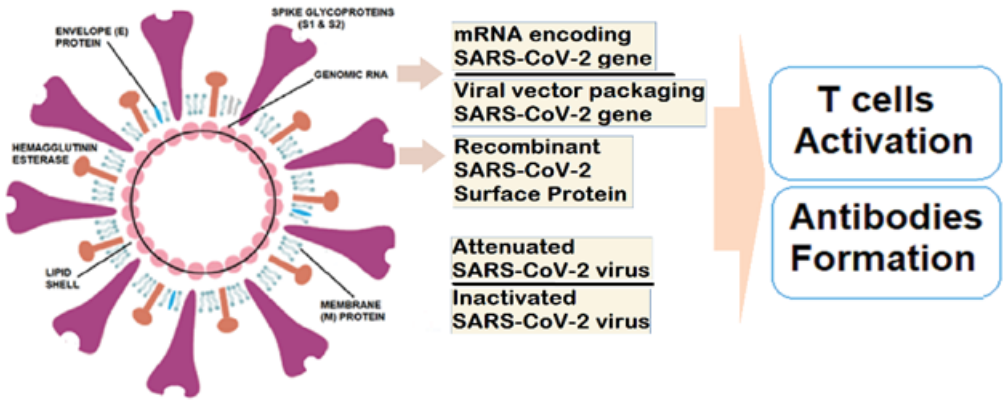

Figure 1: The potential candidates for development of COVID-19 vaccines.

COVID-19 Vaccine platforms	Antigen product and Immunological response
Protein subunits	Antigen fragments - Spike (S) protein or RBD, Weak immunological response Requires repeated vaccination Strong induction
Virus-like particle	Multiple viral antigens, Weak response but greater than protein subunits Requires repeated vaccination Strong induction
Inactivated virus	Multiple viral antigens Weak immunological response Requires repeated vaccination Strong induction
Live attenuated virus	Multiple viral antigens Sustained immunological response Requires only a single delivery Strong induction
DNA-based	Codes for S protein Weaker response than mRNA- based vaccine Requires repeated Vaccination
mRNA-based	Codes for S protein or RBD May be delivered encapsulated with nanoparticle Response depends on adjuvants and formulation May require repeated vaccination
Chimpanzee adenovirus Non-replicating viral vector	Spike protein Unaffected by lack of pre-existing anti-vector immunity Strong with single delivery
Human serotype 26 adenovirus Non-replicating viral vector	Spike protein Response affected by pre-existing anti-vector immunity Requires repeated or heterologous boost vaccination
Human serotype 5 adenovirus Non-replicating viral vector	Spike protein Response affected by pre-existing anti-vector immunity Strong with single delivery

Table 1: COVID-19 Vaccine development technology platforms.

Vaccine development stages and clinical trials:

- Vaccine development stages include - Exploratory or Preclinical Phase - Planning and designing a candidate vaccine.
- Phase I trials test primarily for safety and preliminary dosing in a few dozen healthy subjects.
- Phase II trials – following success in Phase I – evaluate immunogenicity, dose levels and adverse effects of the candidate vaccine, typically in hundreds of people. The phase I and II consist of randomized and placebo-controlled trials.
- Phase III trials typically involve several participants at multiple sites including a control group. It aims to establish the effectiveness of the vaccine to prevent the disease as well as monitoring for the adverse effects at the optimal dose.

Currently, there are more than a hundred COVID-19 vaccine candidates under development, with several of them already in the human trial phase [24]. The WHO is working in collaboration with scientists, business groups, and various health organizations through the Access to COVID-19 Tools (ACT) Accelerator to streamline and speed up the effort. The WHO through the COVAX, which is one of three pillars of the ACT Accelerator, is bringing together governments, global health organisations, manufacturers, scientists, private sector, civil society and philanthropy, to provide equitable access to COVID-19 diagnostics, treatments and vaccines to protect people in all countries.

In a statement issued by the International Coalition of Medicines Regulatory Authorities (ICMRA) and the WHO on 6 November 2020, it was endorsed that ICMRA and WHO are committed to ensure that people in various countries have access to safe and effective health products against COVID-19 as early as possible, while the scientific standards for the evaluation and safety monitoring of treatments and vaccines are rigorously maintained [25]. Simultaneously, they are working to ensure to reduce the risks associated with unproven treatments, and potentially fraudulent and false claims.

Enrolment and plan of clinical trials: The human challenge studies or controlled human infection trials relating to vaccine development, involve an intentional exposure of the participants or test subjects to the vaccine products following preliminary proof of its safety and efficacy in laboratory animals and healthy humans. In this context, the fast-tracking for clinical trials for a COVID-19 vaccine involves compressing the clinical trial period of Phase II and Phase III trials from years to few months. Such challenge studies have been done earlier involving diseases like common flue, typhoid fever, cholera, and malaria which were less deadly than COVID-19.

There is fear that fast-tracking for clinical trials and bypassing typical Phase III research, providing for the accelerated path to license a COVID-19 vaccine may be disastrous and may expose the participants to dangers beyond those considered the anticipated potential side effects. The young adult volunteers are deliberately infected with COVID-19 in a challenge trial conducted and once an infection dose of COVID-19 is identified, the candidate COVID-19 vaccine is tested for effectiveness in preventing infection. Following the challenge, the participants are closely monitored.

Although challenge studies are potentially hazardous for the participants, they are the only way to rapidly produce a vaccine that can be able to prevent the disease in estimated millions of human-beings worldwide and avert significant morbidity and mortality from COVID-19 infection [26]. The clinical COVID-19 challenge studies in healthy people are conducted as per the WHO Guidance Document including scientific and ethical evaluation, public consultation and coordination, selection and informed consent of the participants, and monitoring by independent experts.

The efficacy of the COVID-19 vaccines: The effectiveness of a new vaccine is defined by its efficacy [27]. The minimal efficacy limit set by WHO is 50%. Whereas an efficacy of less than 60% may fail to achieve herd immunity. There are other determinants for the efficacy and factors like genetics, health status (underlying disease, nutrition, pregnancy, and sensitivities or allergies), immune competence, age, obesity, and environmental factors, which may affect the susceptibility to infection and severity of the disease, and response to a vaccine. Further, the viral mutations altering its structure may have impact on the vaccine efficacy [28].

The elpis: Vaccine updates and future scenario for COVID-19

The vaccines in advanced stages of clinical trials –

Out of various COVID-19 vaccine candidates in preclinical and clinical trials, only some are in advanced stages of clinical trials including 11 in phase 3 trials and few vaccine candidates are due for emergency use authorisation (Table 2).

A. Moderna mRNA-1273 Vaccine

The mRNA-1273, the Moderna's mRNA vaccine candidate against the SARS-CoV-2 virus, encodes for a prefusion stabilized form of the Spike (S) protein. Based on data from the results of the Phase 1 study, the dose of 100 mcg is generally well-tolerated across various age groups. Further in the older adults, it has been shown to produce the virus-neutralising antibodies at levels similar to that in the younger subjects [29]. Moderna has revealed no serious safety

Vaccine	Technology platform	Current phase – Clinical studies	Timeline
mRNA-1273 SARS-CoV-2 Vaccine Moderna, Inc.	mRNA-1273 (a mRNA compound) encoding prefusion stabilized S protein	Submitted preliminary data from its Phase III clinical trial on 16 Nov 2020.	Mar 2020 – Late 2022
AZD1222 – University of Oxford, AstraZeneca	Modified chimp adenovirus vector (ChAdOx1)	Phase III (30,000) Completed Phase I-II in the UK, Sao Paulo	May 2020 – Aug 2021
Ad5-nCoV, CanSinoBIO, Beijing Institute of Biotechnology	Recombinant adenovirus type 5 vector	Phase III (40,000) Completed Phase II China and Pakistan	Mar 2020 – Dec 2020
BNT162 - b2 Pfizer, BioNTech, And Fosun	modified mRNA encapsulated in lipid nanoparticles encoding a mutated form of S protein	Phase III (30,000) Completed phase I-II in the US, Germany	Apr 2020 – May 2021
Gam-COVID-Vac – Sputnik V Gamaleya Research Institute of Epidemiology and Microbiology	Non-replicating viral vector	Phase III (40,000) Randomized double-blind, placebo-controlled to evaluate efficacy and safety in Moscow	Aug 2020 – May 2021
CoronaVac Sinovac Biotech Ltd.	Inactivated SARS-CoV-2	Phase III (23,490) Double-blind, randomized, placebo-controlled to evaluate efficacy and safety in Brazil, Chile, Indonesia, and Turkey	Jul 2020 – Aug & Oct 2021

Table 2: The Vaccines in Advanced Stages of Clinical trials.

concerns. An independent board that conducted the interim analysis of the vaccine trials found side effects such as fatigue in 9.7% of participants, muscle pain in 8.9%, joint pain in 5.2%, and headache in 4.5%.

More than 30,000 participants at 100 clinical research sites in the United States are participating in the study, which launched on July 27, 2020, after results from earlier stage clinical testing indicated that the vaccine candidate is well-tolerated and immunogenic. Recognizing the disproportionate impact of the epidemic on minority populations, the study has included 37% of the trial volunteers from racial and ethnic minorities.

The vaccine has been co-developed by the Cambridge, Massachusetts-based biotechnology company Moderna, Inc., and the National Institute of Allergy and Infectious Diseases, a part of the National Institutes of Health. It combines Moderna's mRNA (messenger RNA) delivery platform with the stabilized SARS-CoV-2 spike immunogen (S-2P) developed by NIAID scientists. The vaccine will be manufactured at Visp, Switzerland by its partner Lonza Group, to produce the first doses in December of 2020. Another Lonza's site at Portsmouth, New Hampshire, aims to produce it exclusively for the U.S.A.

Recently, on 16 November 2020, Moderna, Inc. announced that the independent, NIH-appointed Data Safety Monitoring Board for the Phase 3 study of mRNA-1273, has confirmed that Moderna vaccine trial has met the statistical criteria pre-specified in the study protocol for efficacy, with the vaccine having efficacy of 94.5%. Later, on the following day, it announced that the European Medicines Agency human medicines committee has started a review of the vaccine, following the confirmation of eligibility of mRNA-1273 for submission on October 14, 2020.

B. Pfizer-BioNTech COVID-19 Vaccine

The mRNA-based COVID-19 vaccine candidate, BNT162-b2, developed by Pfizer and its German partner, BioNTech SE, has shown 95% effectivity. According to Pfizer data, of the 170 volunteers who contracted COVID-19 during Phase III trials involving over 43,000 people, 162 had received a placebo and only eight received the two-dose vaccine, indicating 95% efficacy of the vaccine.

It is a two-dose vaccine, and the second dose is given 3 weeks later. It takes about 2 weeks for the immune system to make sufficient antibody protection after vaccination. After the first dose, efficacy was 52% and after second dose (3 weeks later), efficacy is 95% (90% - 98%). Further, Pfizer has claimed that the vaccine has consistent efficacy across different ages and ethnicities, and its efficacy in adults over 65 years, who are at particular risk from the virus, is over 94%. Presently, it is not recommended for those under 16 and in pregnant women.

As per the data, the vaccine is well-tolerated and has mild to moderate side effects. About 2% volunteers in the study complained of headache, whereas 2% and 3.7% suffered with fatigue following the first dose and second dose, respectively. Further, there is safety data on about 100 children of 12-15 years of age and about 45% of US trial participants enrolled were 56-85 years old.

On 2 December 2020, Medicines and Healthcare products Regulatory Agency (MHRA) has approved the Pfizer/BioNTech COVID-19 vaccine for widespread use in the UK. This is followed by EUA by health regulatory agencies in Bahrain, Canada, Saudi Arabia, and very recently by USA on 12th December.

Both the Moderna's and Pfizer's COVID-19 vaccines, rely on a technology called messenger RNA, which is being used for the first time to develop a vaccine. The technology is designed to tweak the host cells to make certain proteins for immunological response. These vaccines have specific cold chain and handling requirements. Minus 70 degrees Celsius (-94 °F) is required by the Pfizer vaccine, whereas for Moderna's vaccine it is Minus 20 degrees Celsius. The cold chain requirement, specially for the Pfizer's vaccine can be an obstacle for most Asian countries where high environmental temperature is compounded by poor infrastructure. After reaching a vaccination center, the vaccine is to be thawed and used within five days.

C. AstraZeneca-Oxford COVID-19 Vaccine

The AstraZeneca-Oxford AZD1222 or ChAdOx1 vaccine uses an adenovirus to deliver the gene for the spike protein of SARS-CoV-2 to trigger a robust immune response in adults aged 56-69 and over 70, as per the results of Phase II clinical trials. As per an interim analysis blending two trials of the vaccine in which people received different doses, the efficacy

ranges from 62% to 90%, depending on the dosing strategy. Further, it appears to be well tolerated across all age groups. The AstraZeneca/Oxford vaccine candidate initial efficacy trials were conducted in the United Kingdom and Brazil.

As per the data, the immunogenicity was similar across age groups after a boost vaccination. The immunisation with ChAdOx1 nCoV-19 has shown to result in development of neutralising antibodies against SARS-CoV-2 in almost 100% of participants including older adults without severe comorbidities, with higher levels in boosted compared with non-boosted groups [30]. The adverse reactions to the vaccine are mild, with the most common effects being injection-site pain and tenderness, feverishness, fatigue, headache, and myalgias.

In India, the Phase III trials of the Oxford vaccine (named 'Covishield' in India) are being conducted by the Serum Institute of India. Recently on 20 Nov 2020, it was announced that the vaccine is planned to be tested on a limited basis by administering it to the frontline workers and the elderly. The vaccine is likely to be available for healthcare workers and elderly people by around February 2021 and by April for the general public. The Serum Institute of India has claimed that its vaccine has 90% efficacy.

The AstraZeneca/Oxford vaccine will be relatively cheap, about $3 per dose, and only needs refrigeration temperatures for storage, whereas the mRNA vaccines will be costlier at least $20 per dose and must be kept at sub-zero temperatures.

D. Chinese COVID-19 vaccine

The 'Coronavac', the Sinovac Biotech Ltd.'s COVID-19 vaccine, is a two-dose vaccine. It is under clinical trial involving 9,000 volunteers at Butantan Institute, São Paulo, Brazil. The vaccine is also in the final Phase III trials at University Research Hospital in Kocaeli, Turkey. It was recently declared that Sinovac is likely to publish the efficacy results from its vaccine trials by 15 December, this year.

The vaccine has been offered to Chinese population for emergency use since 17 Oct 2020 onwards, and reportedly, CoronaVac is one of three experimental COVID-19 vaccines China has been using to inoculate around 1 million people under an emergency use programme.

Another Chinese COVID-19 vaccine, with Ad5-nCoV (Recombinant adenovirus type 5 vector) technology platform, named 'CanSinoBIO' is developed by the Beijing Institute of Biotechnology and under Phase III trials involving 40,000 volunteers in China and Pakistan.

E. Russian COVID-19 vaccine

The Russian COVID-19 vaccine, named 'EpiVacCorona' is being developed by Vector State Research Center of Virology and Biotechnology. On 16 Oct 2020, Russia has announced completion of successful clinical trials of EpiVacCorona.

Another Russian COVID-19 vaccine named 'Gam-COVID-Vac', also known as 'Sputnik V', is based on the non-replicating viral vector technology platform to deliver the S protein gene and being developed by the Gamaleya National Research Center for Epidemiology and Microbiology and the Russian Direct Investment Fund (RDIF). Sputnik V vaccine is claimed to be 92% effective at protecting people from COVID-19 according to the interim trial results. The vaccine is to be kept at a temperature of -20 to -70 degrees Celsius.

A combined approach is also being tested in Russia by AstraZeneca, by using a mix of its vaccine with the locally made Sputnik V vaccine in clinical trials. In India, the Russian vaccine, Sputnik V, is under mid- to late-stage clinical trials by Hyderabad-based Dr Reddy's Laboratories in India.

F. Other COVID-19 Vaccines

In India, trials are underway for 'Covaxin', an indigenous COVID-19 vaccine being developed by the Bharat Biotech in collaboration with the Indian Council of Medical Research (ICMR) and National Institute of Virology (NIV), Pune. Covaxin uses a part of inactivated SARS-CoV-2 virus to provoke the immune response. The vaccination schedule consists of two doses of Covaxin for each study participant, administered via intramuscular injection 28 days apart. The Phase III of the human clinical trial of Covaxin is currently underway at All India Institute of Medical Sciences, New Delhi. As stated in a latest communique by Bharat Biotech on 23 Nov 2020, Covaxin is expected to be 60% efficacious, the vaccine can be stored at temperatures between 2 °C and 8 °C, economical at a projected cost ₹ 500-600 (About 8 USD) per dose for the general public and could be ready for rollout by next year, in June 2021.

Another COVID-19 vaccine, **'**ZyCOV-D'**,** developed by the Indian pharma giant, Zydus Cadila, using the DNA based platform using non-replicating and non-integrating plasmid carrying the gene of interest, is currently under phase II of human trials. According to the data from the preclinical stage, the vaccine was found to be immunogenic in multiple animal species and the antibodies produced were able to completely neutralize the wild type virus. In the Phase I clinical trials, the vaccine candidate was found to be safe and well tolerated. The vaccine is expected to be available by March 2021.

Two other vaccine candidates by Sanofi and GSK are not expected to be ready until the end of 2021. The development and trials of the COVID-19 vaccine by CSL and University of Queensland in Australia was abandoned recently due to technical issues about false positive HIV results among subjects involved in early testing.

The British researchers are also studying inhaled versions of COVID-19 vaccine candidates to see if they can deliver a localised immune response in the respiratory tract. An alternative to the common injection in the arm, the spray vaccine is supposed to trigger specific immune responses in airways by mimicking the natural infection of a respiratory virus. China is also set to start trial of nasal spray COVID-19 vaccine. The researchers working on inhaled vaccine plan to utilize some of the unique cellular features of the lungs, nose, and throat.

COVID-19 vaccination - other issues: ***The fast tracks for COVID-19 vaccines:*** Because of the urgency created by the COVID-19 pandemic, the development of various vaccines is on a fast track. Classically, for a vaccine the preclinical stage is about 18 to 30 months, followed by the phase I, II and III, each of them lasting for about two and half years, and the approval followed by production of the vaccine taking a period of another one to two years. For the COVID-19 vaccines being developed urgently, the preclinical stage is short one, followed by phase I and II each compressed to duration as short as 6 months and the phase III shortened to zero month, and the COVID-19 vaccine is foreseen to get an approval for emergency use and start its production simultaneously. The urgency and the haste are likely to involve errors at multiple stages and carry a potential scope for disaster.

Timeline for the COVID-19 vaccination: It is being envisaged that following the emergency authorization for use by US Food and Drug Administration, European Medicines Agency (EMA) or other governing body in a country concerned, the vaccine will initially be offered to people at high risk for the disease and healthcare workers (Dec 2020 -March 2021), followed by those at risk (March 2021 or later), before being available to the general population (July 2021 or later). It is thought that a target to vaccinate 75% population is likely to attain localised herd immunity.

Opposition to the COVID-19 vaccines: The hope of benefiting from the vaccine to the extent that the vaccine may be the only way to tide over and control the COVID-19 pandemic, is accompanied by the likely opposition in public for COVID-19 vaccination, including the vaccine hesitancy. As apparent from various surveys, some people are understandably concerned that the speed of both scientific review and vaccine regulation could compromise safety, despite vaccine developers' and regulators' assurances to the contrary. Vaccine distribution poses another formidable challenge. It is also accompanied by issues such as its cost and who will be paying for it.

In a Medscape reader poll involving 308 UK physicians, it was found that 4 in 10 doctors would not have a COVID-19 vaccine as soon as one is approved by the Medicines and Healthcare products Regulatory Agency in the country. About 56% cited safety concerns, 27% would rather wait, 7% mentioned personal health reasons, and 14% had other reasons. Overall, 59% said vaccination for healthcare staff should not be compulsory [31]. With the growing number of people who oppose the vaccination, their attitudes have also changed over last few months. The polling by Kantar of 1000 people carried out recently on 10-11 November, found that 76% of people in Britain would like to take a vaccine for COVID-19, but the score has fallen since June 2020.

In another European survey, 73.9% participants were willing to get vaccinated against COVID-19 if a vaccine would be available; 18.9% of respondents stated that they were not sure, and 7.2% stated that they do not want to get vaccinated [32]. The common reasons for opposing COVID-19 vaccination, are based on the belief that the vaccine may not be safe (24%), the concern about the side effects (21%), considering the COVID-19 infection not dangerous (14%), rejecting vaccinations as a general principle (11%), and some (8%) not willing to meddle with the course of the Nature [33].

The COVID-19 vaccine nationalism: The COVID-19 pandemic has triggered a global race for development of its vaccine. Further, there has evolved a competition among various countries to ensure the COVID-19 vaccine availability to their citizens. The wealthy governments have invested in vaccine candidates and have made bilateral agreements with developers, resulting in the vaccine doses being reserved.

With over 1.5 billion potential dose purchases and 1 billion confirmed dose purchases, the US alone has signed up for more than 2.6 billion doses. This is followed by the European Union's 1.2 billion doses and another 750 million potential purchase. India has confirmed dose purchases exceeding 1.5 billion, ranking third in terms of the number of COVID-19 vaccine doses committed to procure. Thus, there are already more than 8 billion doses of COVID-19 vaccine currently reserved due to advance market commitments before a clearly confirmed outcome of the effectiveness of a COVID-19 vaccine is released. These factors may hinder the delivery of health innovations to several lower-income countries and potentially leave their populations vulnerable to COVID-19.

The advanced deals made by high-income countries as well as middle-income countries have led to a fear that this may create a challenge for equitable global distribution of coronavirus vaccines [34]. In response to the vaccine nationalism, there has been the creation of the COVAX Facility led by the WHO, which is an international partnership aiming to financially support leading vaccine candidates and ensure access to vaccines for lower-income countries. Seventy-nine higher-income countries are COVAX members. Their governments will help support 92 lower-income countries for affording COVID-19 vaccines.

The populations at greatest risk of serious COVID-19 include people with coexisting health conditions and older adults. A safe and effective vaccine will be an important tool in controlling the global COVID-19 pandemic and the success rate of a vaccine to be introduced for use, as per the WHO recommendations, should show the disease risk reduction by at least 50%. It is estimated about 70% of people must be inoculated to end the pandemic, and Asia alone is home to more than 4.6 billion - or three-fifths of the global population. Further, the Director General of WHO has cautioned that the COVID-19 vaccines alone will not be enough to stop pandemic and that the vaccines should complement the other tools such as universal masking and social distance, not replace them.

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Vaccine related risks and competition: Whereas most of the leading Western vaccines are based on advanced technology platforms such as genetically engineered viral vectors, designer proteins, and snippets of RNA, the China's vaccine candidates and an Indian vaccine candidate make use of the inactivated virus. The inactivated virus, containing a full set of viral proteins, along with an adjuvant effectively alerts the immune system to produce antibody and T cell responses. Unlike the mRNA vaccines, which require to be stored at sub-zero temperatures, the inactivated virus vaccines require ordinary refrigeration.

There are risks related to the COVID-19 vaccines. Many scientists find the inactivated virus vaccines as outmoded, difficult to make in high volume, and potentially dangerous. Further, inactivated COVID-19 vaccines are more likely to trigger antibody enhanced disease in immunized people, in whom ineffective antibodies form immune complexes that clog the lungs and vasculature. This occurred with a vaccine against respiratory syncytial virus given to children in the 1960s, and in animal experiments with vaccines against SARS and MERS. In case of the Adenovirus-based vaccines, pre-existing immunity to Ad5 can attack the vector, leading to a weaker-than-expected antibody response. Further, historically, in 2007, it was feared that the efficacy trials of an Ad5-based AIDS vaccine might have actually raised the risk of HIV infection. Further, the prospect of producing large batches of virus before being inactivated poses certain challenges, as instances of the live poliovirus having escaped from European plants involved in making inactivated polio virus vaccines.

China's vaccine effort is supplemented by the country's dramatic success with aggressive public health measures and testing of entire cities. Further, China is not waiting for the phase III results before widely using its vaccines at home to vaccinate large populations outside of clinical trials. The Coronavac, inactivated virus vaccine and CanSino based adenovirus 5 (Ad5) incorporated with the S protein have been offered to Chinese population for emergency use since 17 Oct 2020 onwards and as against the doubting attitudes and mistrust toward COVID-19 vaccines in the United States and European countries, people in China have already lined up to receive the experimental vaccines even before their value and safety have been proved.

China is doing the clinical trials and brokering vaccine deals in various countries including the Arab and South American countries. The China's COVID-19 vaccines manufacturers claim to produce billions of doses by next year for the countries not having access vaccine because of vaccine nationalism and the countries, like Brazil, Mexico, Chile, Argentina, Turkey, Indonesia, and Pakistan, that have hosted China's efficacy trials in procuring a secure vaccine supply.

With the pandemic controlled at home, China looks forward to supplying its COVID-19 vaccines to various countries around the world. During October, China joined the COVID-19 Vaccines Global Access (COVAX) Facility, led by WHO, CEPI, and Gavi, the Vaccine Alliance, to make sure that the products are safe and effective and available to higher income as well as low-income countries. The COVAX has not, so far, received support from the United States or Russia [35].

Vaccine related injury and compensation: As early as 2005, the International Federation of Pharmaceutical Manufacturers and Associations demanded that manufacturers be granted protection from lawsuits associated with vaccine-related adverse events if they were going to participate in pandemic responses. Following which, in the United States, the Public Readiness and Emergency Preparedness Act was passed in the background of clinical trials of avian influenza vaccine, providing manufacturers immunity from lawsuits related to injuries caused by vaccines, with narrow exceptions, in the event of a declared public health emergency. In the current situation the same kind of immunity is being claimed by the vaccine manufacturers [36].

About 25 countries have no-fault vaccine-injury compensation systems for routine immunizations and required changes could be made to policies related to funding, proving injury, and distributing compensation. Other countries need to agree to appropriately provide legal immunity and indemnify the WHO, donors, manufacturers, and health care workers who vaccinate people. Simultaneously, there is required a mechanism for efficiently handling a high volume of claims from throughout the world.

To meet this, it is being explored that the COVAX Facility may establish a procedure for compensating people who may suffer from severe adverse events related to the vaccination. Further, the international body for compensation based at the COVAX Facility may be practicable solution to facilitate the procurement of COVID-19 vaccines while ensuring that vulnerable people are able to seek compensation for injuries, and it could as well set a precedent for future vaccination campaigns.

Conclusion: The limitations

The concerns and hesitancy

Immunological response to the vaccines: Understanding immune memory to SARS-CoV-2 is critical for improving diagnostics and vaccines, and for assessing the likely future course of the pandemic. The various components of immune memory of SARS-CoV-2 tend to persist for about 6 months [37]. The S-specific memory B cells are more abundant at 6 months than at 1 month. Whereas the SARS-CoV-2-specific CD4+ T cells and CD8+ T cells decline with a half-life of 3-5 months.

The neutralising antibodies appear to be the only component of immune response that can provide protection from the infection. The spike IgG titres show a modest decline in 6 to 8 months. But the magnitude of the antibody response against SARS-CoV-2 is highly heterogenous between individuals. Immunization studies in non-human primates have indicated that circulating neutralization titres of 200 or more may provide sterilizing immunity. Further, the presence of sub-sterilizing neutralizing antibody titres at the time of exposure to infection may blunt the size of the initial infection and may contribute to limit the disease severity.

Efficacy *vs.* exaggerated immune reactions: The confirmation of the correlation between antibody titres and protection against COVID-19 can only be possible through a large clinical efficacy study. In the meantime, the assays for measuring antibody may fill the gap but their validity needs to be ascertained. There is an uncertainty relating to the expected efficacy. It is being projected, depending on the profiles observed for other viral vaccines, that the vaccine's efficacy against severe COVID-19 may be higher than efficacy against mild disease.

But there is another aspect of the immunological response to the vaccine. Although the antibody production by a potential vaccine is intended to neutralize the COVID-19 infection, it is feared that the vaccine may have an opposite effect by causing antibody-dependent disease enhancement (ADE), which might trigger the cytokine storm in case the person

is infected by the virus in future, after the vaccination [38]. Following inoculation of inactivated whole-virion coronavirus vaccine, the antibodies targeting S protein, may involve the Fc receptor (FcR)-expressing cells, a phenomenon well documented with flaviviruses, leading to ADE. The technology used for vaccine, its dose, timing of repeat vaccinations for the possible recurrence of COVID-19 infection, and elderly age are factors related to the risk and extent of ADE.

The vaccine hesitancy and concerns: The rapid development and urgency of producing a vaccine for the COVID-19 in view of the raging pandemic may increase the risks and failure rate of delivering a safe and effective vaccine. There are indications that the potential success rate may be only 10% for various COVID-19 vaccine candidates under development [39]. It is, thus, important to continue the developmental research for an effective and safe SARS-CoV-2 vaccine.

On the other hand, at least 10% of the people in different surveys perceive the COVID-19 vaccines as unsafe or unnecessary and consider refusing the vaccination. This public perception has been called vaccine hesitancy [40]. Such behaviour can increase the risk of further viral spread that could lead to future COVID-19 outbreaks. As per a survey in the United States about 67% or 80% of people would accept a new vaccination against COVID-19, with wide disparity relating to education level, employment status, and racial and geographical background [41]. Similar and comparable findings have been documented in a study from the United Kingdom [42].

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Chapter 4: Evolving Patterns in COVID-19: The Virus, its Variants and Infectivity-cum Virulence

Background

The virus, variants and pandemic: Despite ongoing research and discoveries relating to COVID-19 as a disease and SARS-CoV-2 as its causative organism, the emerging patterns remain an enigma to significant extent. The pandemic continues to persist over a year with outbreaks and re-emergence involving of newer regions and population groups. While we continue to explore the agent factors, disease transmission dynamics, pathogenesis and clinical spectrum of the disease, and therapeutic and vaccine modalities, the grievous nature of the disease is an evolving further with the genomic mutations and pathophysiological alterations.

The emerging SARS-CoV-2 variants: Understanding the nucleotide variations in the virus genome provides a useful insight for the changing face of the disease and propagation of the pandemic. The emerging mutations can be interpreted as a strategy through natural selection to facilitate extensive spread of the viral infection. Of late, there is recurrent emergence, accumulation, and onward transmission of various mutations. Among the Current major SARS-CoV-2 lineages A, B, B.1, B.1.1, and B.1.177, the lineage - B.1.1.7 is of present concern.

Challenges posed by the mutations: In general, the D614G mutation leads to open conformations, increases density of the S protein and disease infectivity by facilitating the viral entry into the host cells. Another variant, named VUI-202012/01 or B.1.1.7 identified in the UK, harbours multiple mutations which have been correlated with a significantly increased infection transmission rate during recent months. A new entrant, the South African variant carries a high infectivity and accompanied by probable high disease severity. There are salient potential consequences of emerging variants including rapid transmission, enhanced disease severity, and diagnostic failure as the variants may evade detection through certain diagnostic tests. Further, they may decrease susceptibility to antiviral drugs and monoclonal antibodies and evade natural or vaccine-induced immunity.

Conclusion: Dealing with the variants: The WHO is working with various countries to amplify and adapt the current surveillance systems to evaluate potential virus variations through ongoing systematic clinical and epidemiologic surveillance. There is need to establish genomic sequencing capacity and access to the sequencing services. Simultaneously, the disease control measures, and intensive public health response are needed to be strengthened to curtail the increased transmissibility associated with the emerging SARS-CoV-2 variants.

Introduction: The virus and pandemic

COVID-19 as a disease and SARS-CoV-2 as its causative organism, continue to remain an enigma. The COVID-19 pandemic continues to persist over a year with outbreaks and re-emergence and involvement of newer regions and population groups. COVID-19 has changed the ways of life for mankind, damaged economies, and altered sociocultural structure. Moreover, it appears that the SARS-CoV-2 virus is here to stay for the foreseeable future [1] and continue to create further deterioration and havocs.

Mutations and variants of SARS-CoV-2

While we continue to explore the agent factors, disease transmission dynamics, pathogenesis and clinical spectrum of the disease, and therapeutic modalities, the grievous nature of the disease is evolving further with the genomic changes in the virus and pathophysiological alterations and clinical manifestation. The future of the disease, of course, is stated to depend on various known and unknown factors, which include the effects of seasonality on the disease transmission and spread. At the individual level, it is to depend on the degree and duration of effective immune response to the disease, and at the cohort and societal level on the life-style choices made by individuals and measures enforced by the governments.

Further, the pandemic's course will also depend on the availability of a COVID-19 vaccine, its efficacy and duration of its protective immune response. Concerning the latter, the significance of viral mutations should also be highlighted and explored further. There are serious challenges posed by SARS-CoV-2 virus and COVID-19 as the disease that are further increased by mutations and evolution of variants with enhanced infectivity and probably virulence. Looking forward, let us hope that the today's unmet challenges are resolved in near future [2].

The viral variants *vs.* strains

Being an RNA virus, SARS-CoV-2 has a steady rate of mutations despite presence of the spell-checker, nsp14 protein acting as 3′-5′ exoribonuclease. It is expected and has been observed that the virus accumulates mutations over time. With the mutations, the variants may lose or gain infectivity and virulence. As feared, enhancing the infectivity and virulence due to mutations may pose a heightened challenge for COVID-19 therapeutic and preventive modalities, including vaccines.

There is no universally accepted definition for the terms strain, or variant. According to van Regenmortel, et al. a virus strain is a variant of a given virus that is recognizable because it possesses some unique phenotypic characteristics that remain stable under natural conditions [3]. Whereas the unique phenotypic characteristics and biological properties for a virus strain are different from the compared reference virus, such as the antigenic properties, host range or the disease manifestations it can cause [4]. Further, a virus variant with a simple difference in genome sequence should not be given the status of a separate strain since it lacks a distinct recognizable viral phenotype [5]. Mutations in SARS-CoV-2 are common and over 4,000 mutations have been detected in the spike (S) glycoprotein alone, according to the observations by COVID-19 Genomics UK (COG-UK) Consortium.

SARS-CoV-2 clades and lineages

There have been identified several thousand variants of SARS-CoV-2. These variants have been placed under larger groups called clades. Due to lack of uniformity, there have been proposed several nomenclatures for SARS-CoV-2 clades. As of December 2020, the Global Initiative on Sharing All Influenza Data (GISAID) in reference to SARS-CoV-2 or hCoV-19, has identified seven clades - O, S, L, V, G, GH, and GR [6]. Whereas Nexstrain has, as of December 2020, found five - 19A, 19B, 20A, 20B, and 20C [7]. In this reference, Guan et al. have recently named five global clades - G614, S84, V251, I378 and D392 [8]. Further, Rambaut, et al. have proposed the term 'lineage' and identified five major lineages A, B, B.1, B.1.1, and B.1.177 [9]. Both the Nextstrain and the GISAID clade nomenclatures, in general, aim at a broad categorisation of globally circulating diverse SARS-CoV-2 variants, whereas the lineages nomenclature by Rambaut, et al has been related to the disease outbreaks and re-emergence.

Trying to simplify the issue, a WHO document has identified six major clades with 14 subclades [10]. The largest clade is D614G clade with five subclades. Within D614G clade, D614G/Q57H/T265I subclade forms the largest subclade with 2391 samples. The second largest major clade is L84S clade, observed among travellers from Wuhan and consists of 1662 samples with 2 subclades. The L84S/P5828L/ subclade is predominantly observed in the United States. Whereas G251V frequently appears in samples from the United Kingdom, Australia, the United States, and Iceland. The remaining two clades D448del and G392D are smaller and without substantial subclades at this point.

Emerging SARS-CoV-2 variants

Mutations and variation in SARS-CoV-2: Understanding the nucleotide variations in the SARS-CoV-2 genome provides a useful insight for evolution of the disease and propagation of the pandemic [11]. The early variations have made their way almost unnoticed as the virus spread around the world. Whereas most variations or mutations have no impact on the viral ability to transmit or cause disease, certain mutations appear to have impact on transmissibility, infectivity, or lethality. Some of these mutations have possibly arisen as a result of the virus evolving from immune selection pressure in infected individuals and are more prevalent in patients with mild than those with severe disease. In general, the mutations can be interpreted as a strategy through natural selection to facilitate extensive spread of the viral infection.

As such the SARS-CoV-2 virus has a low mutation rate by virtue of the nsp14 protein acting as 3′-5′ exoribonuclease on both single-stranded and double-stranded RNA during the viral replication cycle [12]. Still its large genome appears to facilitate recombination, insertions, and deletions. Andrés, et al. have noted that the viral S protein accumulates deletions upstream and close to the S1/S2 cleavage site [13]. Further, SARS-CoV-2 can resort to RNA viral evolution through recombination (synthesis of chimeric RNA molecules from two different progeny genomes) and reassortment (the packaging within a single virion of genomic segments from different progeny viruses).

In general, the single nucleotide variations (SNVs) as SARS-CoV-2 Spike amino acid replacements in the receptor binding domain (RBD) occur relatively frequently [14]. There is recurrent emergence and significant onward transmission

of a six-nucleotide deletion in the S gene resulting in loss of two amino acids labelled as ΔH69/ΔV70. This deletion often co-occurs with the receptor binding motif amino acid replacements N501Y, N439K and Y453F. As such, these deletions have been found in a small percentage (2.2%) of the samples [15]. Among the Current major SARS-CoV-2 Lineages A, B, B.1, B.1.1, and B.1.177, the lineage - B.1.1.7, of present concern. First sequenced in the UK on 20 Sep 2020, it is spreading to other countries and has been discovered in Denmark, the Netherlands, Italy, Israel, Australia, Hong Kong, Singapore, Japan and the USA. The other countries are being increasingly involved.

Using the complete sequences of 1,932 SARS-CoV-2 genomes, six types of the variants have been identified. The 13 signature variations in the form of SNVs in protein coding regions and one SNV in the 5′ untranslated region (UTR) provide interpretation for the six types (types I to VI). The type VI, characterized by the four signature SNVs C241T (5′UTR), C3037T (nsp3 F924F), C14408T (nsp12 P4715L), and A23403G (Spike D614G), with strong allelic associations, first reported in China, has become the dominant type world over. Out of these, C241T is in the 5′ UTR appears to be of uncertain significance. The other three SNVs, 3037T-14408T-23403G characterising the increasing frequency of the type VI, in majority of samples from various regions indicate a possible fitness gain for the virus. Further, it has been noted that the variants missing one or two of these signature SNVs fail to persist or wiped out by other evolutionary more fit variants [16]. Taking a note of the major mutations, their lineages and effects on disease transmissibility is important to understand the changing face of the pandemic (Table 1).

Emergence of the D614G variant: The genomic analysis of various samples for SARS-CoV-2 from several regions has found an increased proportion of some particular variants. One such variant is the D614G mutation in the C-terminal end of the S1 domain and in proximity to the S2 subunit. In short called the G variant, it has increased in prevalence during the pandemic, probably after initially arising in China and then spreading to Italy in January and later globally to become the dominant form in the pandemic [17]. The SARS-CoV-2 G variant is part of the G clade by GISAID and the B1 clade by the Phylogenetic Assignment of Named Global Outbreak LINeages (PANGOLIN).

The variant is associated with the faster viral transmission and harbouring and discharge of higher viral loads by virtue of higher binding to the ACE2 receptor and higher protein stability [18]. In addition, it is associated with reduced S1 Shedding. The presence of glycine at 614, thus, appears to improve the S-Protein Stability and Increase its incorporation into virions. Further, the structural analyses have revealed that the RBD of the D614G form of the spike protein is more likely to assume an “open” conformation than the RBD of the ancestral D614S form, resulting in an improved ability to bind to the hACE2 receptor. Furthermore, the higher viral load with D614G is consistent with epidemiological data suggesting enhanced infectivity associated with D614G [19].

Mutation	Lineage	Effects
D614G	B.1	Moderate effect on transmissibility
A222V	B.1.177	Fast growing lineage. No mutation effects documented
N439K	B.1.141 B.1.258	Eludes some mAbs Enhanced affinity for hACE2 receptor binding
Δ69-70	B.1.1 B.1.258	Evades immune response Diagnostic failure in assays targeting S gene
N501Y	B.1.1.7	Fast growing lineage Enhanced affinity for hACE2 receptor binding
N501Y + Δ69-70	B.1.1.7	Fast growing lineage Evades immune response Enhanced affinity for hACE2 receptor binding
N439K + Δ69-70	B.1.256	Evades immune response Enhanced affinity for hACE2 receptor binding
Y453F	B.1.1 B.1.1.298	Eludes some mAbs Enhanced affinity for hACE2 receptor binding

Table 1: Major mutations, lineages and effects on disease transmissibility and course.

Studies in human respiratory cells and in animal models demonstrated that compared to the ancestral virus, the variant with the D614G substitution has increased infectivity and transmission. With the studies in hamsters infected with D614S and D614G variants, Plante et al. have documented that the D614G variant replicated to higher titers in nasal-wash samples early after infection than the ancestral D614S variant. These findings suggest increased viral fitness for D614G in the major upper airway compartment potentially associated with enhanced transmission [20]. Experimentally, the D614G variant was found to be equally sensitive to neutralizing antibodies and did not cause more severe disease than the ancestral variant in hamsters, an observation supporting current findings in humans [21].

As shown, the SARS-CoV-2 virus with the D614G mutation is more infective in laboratory tests. But the results of lab tests cannot be directly extrapolated to the real-world occurrence as a lot of additional factors are involved in human transmission, including host-pathogen interactions, host genetics and other epidemiological considerations. Further, the mutation does not appear to have impact on the virulence and the SARS-CoV-2 virus with the D614G substitution does not cause more severe illness or alter the effectiveness of existing laboratory diagnostics, therapeutics, vaccines, or public health preventive measures.

In general, the D614G mutation leads to open conformations, increases density of the S protein and disease infectivity by facilitating the viral entry into the host cells [22]. Further, this mutation It is often accompanied by other mutations involving parts of the SARS-CoV-2 genome. Epidemiologically, the rapid spread of D614G was first spotted in early samples collected from China and Germany. In due course, it has become the dominant strain across the European continent, Australia, Canada, and parts of the United States, and probably rest of the world regions. It appears that D614G represents a more transmissible form of SARS-CoV-2, which has emerged as a product of natural selection [18]. There is a potential concern that a similar situation may occur with the VUI-202012/01 or B.1.1.7 variant.

Emergence of VUI-202012/01 variant: Another variant, named VUI-202012/01 (Variant Under Investigation, year 2020, month 12, variant 01) or the phylogenetic cluster named lineage B.1.1.7 has been identified through viral genomic sequencing in the UK. It has 17 mutations that may lead to a conformational change in the shape of the virus including the S protein. Out of the acquired 23 changes - 14 non-synonymous (amino acid [AA] altering) mutations, 6 synonymous (non-AA altering), and 3 deletions. The deletions include a double 69/70 deletion and P681H - near the S1/S2 furin cleavage site, in the S protein, and ORF8 stop codon (Q27stop) - in open reading frame 8. Apart from this, there are multiple S protein mutations such as N501Y, A570D, D614G, Y453F, T716I, S982A, D1118H as well as mutations in other genomic regions. Recently a distinct phylogenetic cluster (named lineage B.1.1.7) was detected within the COG-UK surveillance dataset. This cluster accounts for an increasing proportion of cases in various parts of England [9,23]. Further, the preliminary analyses in the UK suggests that the UK variant B.1.1.7 having multiple mutations is significantly more transmissible than previously circulating variants, with an estimated potential to increase the reproductive number (R) by 0.4 or greater with an estimated increased transmissibility of up to 70% [24]. As reported by the UK, there is no clear epidemiological link to animals for VUI 202012/01 [25].

The variant was first detected during October 2020 in the UK from a sample taken in Sep 2020. The backwards tracing using genetic evidence suggests this new variant first emerged in September 2020 and then circulated at low levels in the population until mid-November, and thereafter it has spread alarmingly by mid-December. It has been correlated with a significantly increased infection transmission rate in the UK, having a 'selection coefficient' of 0.70 (70%), with a generational interval of 6.5 days, and being 74% more transmissible [26]. The cases with the new variant have been detected from various European countries including Denmark, Netherlands, and Belgium. The impact of suddenly increasing spread of the VOC 202012/01 variant was not made out till late-December [27].

The emergence of VUI-202012/01 has certain likely implications:

There is a probability of a wider spread of the new virus variant across the European nations and continents.

The potential impact on SARS-CoV-2 diagnostics - The UK reports that the deletion 69-70 in the spike protein of the variant causes a negative result from S-gene RT-PCR assays applied in some laboratories in the UK. This specific mutation has occurred many times in different countries and is geographically widespread.

The potential impact on severity of disease in a population or groups - The available information regarding severity of the new virus variant is limited. To date, there is no indication of increased disease severity observed related to the variant, but the assessment is presumptive as most of the reported cases are people under 60 years old, who are less likely to develop severe symptoms.

Rapid increase of a SARS-CoV-2 variant with multiple spike protein mutations in the UK, endorses significantly increased infectivity. None of the previously described SARS-CoV-2 variants have shown this property.

Frequency of reinfections - The mutations observed in the new variant are related to the RBD and other surface structures, which may alter the antigenic properties of the virus. Based on this observation, it is likely that reduced neutralisation by antibodies may be seen. But so far there is no evidence for increased risk for reinfection.

The potential impact on vaccine effectiveness is a worrisome issue. It is being speculated based on limited studies that the efficacy COVID-19 vaccines may remain like the ancestral SSARS-CoV-2 virus.

The VOC-202012/01 variant, also known as lineage B.1.1.7, is notable for a high number of mutations it contains. In all it involves 23 mutations: 13 non-synonymous mutations, 4 deletions, and 6 synonymous mutations [9]. Whereas 17 mutations alter proteins, the remaining six do not. The most important change in VOC-202012/01 is N501Y, the change from asparagine (N) to tyrosine (Y) at amino-acid position 501, inside the receptor-binding motif (RBM) part of the RBD [28]. Another feature in the variant, the Q27stop mutation renders the ORF8 protein inactive, which is an immunoglobulin (Ig)–like protein modulating the pathogenesis by mediating major histocompatibility complex I (MHC-I) degradation and suppressing type I interferon (IFN)–mediated antiviral response and associated to milder disease and better prognosis.

As such the mutations in the RBD can change antibody recognition, ACE2 binding specificity, and the infectivity. Thus, the mutation N501Y affects the receptor binding affinity of the spike protein and alone or in combination with the deletion at 69/70 in the N terminal domain (NTD) enhances the transmissibility. An examination of the global GISAID SARS-COV-2 sequence database shows that this N501Y mutation has been circulating sporadically earlier outside the UK, in Brazil in April, Australia in June-July, and in USA in July 2020 [29]. The same mutation being independently selected several times appears to imply its fitness for the virus.

Presently the preliminary epidemiologic indicators suggest the B.1.1.7 variant is associated with higher viral loads in respiratory tracts and increased transmissibility associated at least partly with the N501Y mutation [30]. However, there is no indication of change in the disease severity as measured by length of hospitalization and 28-day case fatality. But on a careful note, a clinical study has documented that the variant in a patient was less susceptible to convalescent plasma than wild-type virus [14]. Thus, there are potential concerns that the B.1.1.7 variant might lead to more severe disease or even evade vaccine-induced immunity.

The B.1.1.7 variant is reckoned to have spread in the UK following its probable emergence during September 2020 and rapidly replaced other variants, and since then has been detected in numerous countries around the world, including the US, Canada, Australia, and Japan.

The South Africa 5O1Y.V2 variant: Another new variant, the South African lineage, has been detected as rapidly spreading in the Eastern Cape, Western Cape, and KwaZulu-Natal provinces in South Africa. This variant was first identified in Nelson Mandela Bay, South Africa, in samples dating back to the beginning of October 2020, and cases have since been detected outside of South Africa including Zambia in late December 2020.

The variant bears the N501Y mutation and accompanied with a high viral load and increased transmissibility and has largely replaced other SARS-CoV-2 variants in the region. Currently, there is no evidence of this variant being associated with more severe disease or worse outcomes. The variant has additional mutations, E484K and K417N, in the RBD in the S glycoprotein and is associated with immune escape [31].

This variant named 20C/501Y.V2 or B.1.351 lineage has emerged independently of the B.1.1.7 lineage and shares some mutations with the B.1.1.7 lineage. It has multiple mutations in the spike protein, including N501Y but does not contain the deletion at 69/70. It is feared that out of the two N501R variants, V1 – the English and V2 - the South African, the latter with 2 other mutations, K417N and E484K, in the RBD of the S protein could be more infectious. The variant can infect younger people with no underlying health conditions and may resist the vaccine [32].

While the variants D614G, 202012/01 and 501Y.V2, and other variants appear to influence the disease infectivity, they do not appear to cause changes in clinical presentation or severity (Table 2). However, the higher case incidence may lead to an increase in COVID-19 hospitalizations and deaths and more intensive public health measures may be required to control transmission of these variants.

The Variants	Associated mutations	Effects on disease	Remarks
D614G	Substitution of aspartic acid (D) with glycine (G), due to single mutation in RNA codon	increased infectivity and transmission. Higher viral load and discharge	Dominant variant in most countries and in 5 out of 6 WHO regions
VUI-202012/01 or B.1.1.7	Multiple S protein mutations – N501Y, A570D, D614G, Y453F, T716I, S982A and D1118H Mutations in other genomic regions.	Increased infection transmission, Disease severity? Effect on vaccine?	First detected in the UK, fast spreading to other countries
South African 5O1Y.V2 or B.1.351	N501R, K417N and E484K	Increased infectivity and ?disease severity, May evade vaccine response	First detected in South Africa, spreading to other African countries
Danish Cluster 5	Multiple mutations including Y453F Spread from minks to human	Decreased sensitivity to neutralizing antibodies	The variant has now become extinct
Nigerian Variant or B.1.207	S protein mutation, S:P681H - involving S1/S2 furin cleavage site	No evidence of increased transmission rate	Accounts for only about 1% SARS-CoV-2 genomes
The escape variants	Prominently encountered, Escape variant S:N440K in India	One of 19 escape variants (2.1% SARS-CoV-2 genomes)	Evolved during recent months
ORF8 stop codon	The mutation in open reading frame 8	The effect seems highly variable	Emerged and disappeared earlier

Table 2: Variants associated with concern and the variants to be watched.

The Danish "Cluster 5" variant: The Cluster 5 variant, also referred to as ΔFVI-spike, was identified by the Danish State Serum Institute (SSI) during August 2020. It was discovered in Northern Jutland, Denmark, and is believed to have been spread from minks to humans via mink farms. The variant has multiple mutations and preliminary studies indicated that the variant may potentially result in reduced virus neutralization in humans, which could potentially decrease the extent and duration of immune protection following natural infection or vaccination. As per the WHO communique also the cluster 5 has been found to have a moderately decreased sensitivity to neutralizing antibodies [33].

Following identification, extensive investigation and surveillance, Danish authorities have identified only 12 human cases of the Cluster 5 variant in September 2020. Subsequent to culling of the mink population, lockdown. and travel restrictions, and mass-testing the variant was held from spreading further, and SSI announced on 19 November 2020 that cluster 5 in all probability had become extinct [34].

Other isolated and minor mutations: B.1.207 variant: The P681H, a S protein mutation involving the S1/S2 furin cleavage site, has been recently reported from Nigeria. The P681H mutation is shared in VOC-202012/01, but has emerged spontaneously several times earlier also, and there is no evidence to indicate it contributing to increased transmission of the virus in Nigeria [35]. Earlier the analysis of samples collected in Aug 2020 and sequenced at the African Centre of Excellence for Genomics of Infectious Diseases (ACEGID), Nigeria, identified two SARS-CoV-2 sequences that share one non-synonymous SNP in S protein in common with this lineage. At the moment, only about 1% of the SARS-CoV-2 genomes from Nigeria share any of the 17 protein-altering variants from the UK lineage of concern (B.1.1.7) and other mutations such as the N501Y, A570D, and the HV 69 - 70 deletion in S protein have not been detected in Nigeria.

ORF8 stop codon (Q27stop): This mutation is not in the S protein but involves the open reading frame 8. The mutations akin to this, have occurred in the past. In Singapore, one strain with this type of mutation emerged and disappeared. This is in line with the understanding that SARS-CoV-2 ORF8 is poorly conserved among coronaviruses. Among accessory genes, open reading frame 8 (ORF8) stands out by being highly variable and may be related with the virus ability to spread [36].

S:N440K variant: The CSIR Institute of Genomics and Integrative Biology (CSIR-IGIB) team in India has identified 19 genetic variants of the SARS-CoV-2 in India which have evolved to evade neutralising antibodies. Out of the 19 immune escape variants in India, one in particular known as S:N440K variant has been reported in 2.1% of the gene sequences in

India [37]. This variant appears to have evolved during the recent months. It has been associated with a case of reinfection in a 28-year-old female healthcare worker and possibly confers resistance to neutralising antibodies.

The challenges posed by SARS-CoV-2 mutations

The genomic analysis of SARS-CoV-2 variants: Of the 2969 missense variants, 1905 variants are found in ORF1ab, which is the longest ORF occupying two thirds of the entire genome. ORF1ab is transcribed into a multiprotein and subsequently cleaved into 16 non-structural proteins (NSPs). Of these proteins, NSP3 has the largest number of missense variants among ORF1ab proteins. Of the NSP3 missense variants, A58T was found to be the most common followed by P153L. The most common variants were the synonymous variant 3037C > T (6334 samples), ORF1ab P4715L (RdRp P323L; 6319 samples) and SD614G (6294 samples). Further, they occur simultaneously in over 3000 samples, mainly from Europe and the United States [10].

The most common clade identified is the D614G variant, located in a B-cell epitope with a highly immunodominant region and may therefore affect vaccine effectiveness. Although amino acids are quite conserved in this epitope, there have been identified 14 other related variants [38]. Almost all variants with D614G mutation, in addition have a mutation in the protein responsible for replication (ORF1ab P4715L; RdRp P323L), which might affect replication speed of the virus. This protein being the target of the anti-viral drugs such as remdesivir and favipiravir, the probability of mutations may lead to emergence of the treatment resistant type. Whereas the mutations in the RBD may not be selected further as they are likely to reduce receptor binding affinity which would decrease the virus fitness [39]. V483A and G476S are primarily observed in samples from the United States, whereas V367F is found in samples from China, Hong Kong, France, and the Netherlands. The V367F and D364Y variants have been reported to enhance the structural stability of the S protein and facilitate efficient receptor binding.

All viruses, including SARS-CoV-2, change over time, most without a direct benefit to the virus in terms of increasing its infectiousness or transmissibility, and sometimes limiting propagation. The mutations alter RNA virus pathogenesis, virulence, transmissibility, or a combination of these. The potential for virus mutation increases with the frequency of infections. Therefore, reducing transmission of SARS-CoV-2 by disease control measures as well as avoiding introductions to animal populations, are critical aspects to the global strategy to reduce the occurrence of mutations with negative public health implications. Further studies are required to understand the impact of specific mutations on viral properties and the effectiveness of diagnostics, therapeutics. and vaccines. Though, it is thought that a COVID-19 vaccine is likely to work because vaccines use multiple targets to elicit an immune response [40].

The genome data outlines that two SARS-CoV-2 virus samples collected from anywhere in the world differ by an average of just 10 RNA letters out of 29,903 [41]. Thus, there is a sluggish mutation rate. But in spite of this, the researchers have catalogued more than 12,000 mutations in SARS-CoV-2 genomes. Most of the mutations appear not to affect the virus's ability to spread or alter the disease severity. In fact, the mutations altering the viral structural proteins are more likely to harm a virus and make it extinct rather than improve it. On the other hand, an insignificant mutation can downgrade the immune response. Further, various mutations having no major impact might in future course of the disease and pandemic may accumulate to alter the infectivity and disease severity [42].

The COVID-19 pandemic in Africa and Asia: Till now the African countries have reported far fewer cases and deaths from COVID-19 than predicted. As of 22 November 2020, the continent of Africa, comprising 1.3 billion people, had recorded 2,070,953 cases of COVID-19 and 49,728 deaths [43]. This represents about 3.6% of total global cases and is far too low than the predicted up to 70 million Africans may be infected with SARS-CoV-2 with more than 3 million deaths by June 2020 [44]. A sero survey study for measuring the occurrence of SARS-CoV-2 antibodies in blood donors in Kenya has highlighted that the incidence of SARS-CoV-2 infection is much higher than expected from case numbers [45]. Similarly, in October 2020, Mozambique reported less than 3000 confirmed cases of COVID-19; however, sero-surveys have found the actual transmission much higher [46].

This suggests that there may be more infections than documented. A similar scenario exists for other developing countries including India, where the Sero-survey study by the Department of Science and Technology (DST), has shown about 90 missed cases for every case detected. The DST has developed the COVID-19 India National supermodel indicating that 60% of the Indian population may have already been infected and recovered. The estimate was arrived based on mathematical and statistical model to map the trajectory of the virus. It does not denote a failure of detection system but may indicate that the effect of the disease has not been severe [47].

The Ill-defined and short-lived immune response: In the experimental studies using animals and cell cultures, along with the latest molecular techniques, and in small human clinical studies, the immune response to SARS-CoV-2 has been recognised [48]. Further, the animal studies in mice, primate studies in monkeys and human clinical studies have documented that those who received one of the experimental RNA vaccines, produced antibodies that proved more potent at blocking G viruses than D viruses [49]. With the G variant now dominant globally, these findings are encouraging. So far, various SARS-CoV-2 variants do not stop the immune system from developing neutralizing antibodies that recognize the virus.

The researchers are, though, wary of the potential of SARS-CoV-2 to evade immunity and antigenic responses like the HIV. There exists a probability that the virus may acquire mutations that either evade the immune response or alter its susceptibility to antibodies and immune cells. Further, it is not certain whether the immunity will be effective or lasting. A related unanswered question is whether a vaccine will provide adequate protection, so that those who have recovered from COVID-19 or vaccinated can return to pre-pandemic behaviours. Furthermore, the reinfections have been documented to occur [50]. But so far neither the frequency nor the elements of the immune response associated with reinfection have been adequately researched and well documented.

The effect of mutations on COVID-19 vaccines: Presently, the researchers as well as clinicians are most concerned about several mutations occurring in group in the S protein. The accumulation of multiple mutations as in the British variant or South African variant is more of a concern and could potentially impede immune protection. Another unresolved issue is the concern that the mutations can have far-reaching consequences for the human health in form of delayed disease complications.

There is a chance that vaccines currently being administered in the country may not provide sufficient immunity against new strains emerging in both the UK and South Africa. Though, there is no evidence that the vaccines currently being administered will not be able to protect against these new variants. Such mutations may make the virus less susceptible to the immune response triggered by the vaccines. It is being conjectured that even if the vaccine is not effective against the new variants, COVID-19 vaccine manufacturers might be able to make changes to the vaccine to add protection against the emerging variants. Scientists believe such changes, if needed can be made in about six weeks' time.

The staggered COVID-19 vaccine dosing in the UK: Facing rising infections and the prospective ever-tightening lockdowns, the United Kingdom has reportedly decided to delay the second dose of both the Pfizer-BioNtech and Oxford-AstraZeneca vaccines. The proposed change from 21 days between doses to 12 weeks, will allow more people to get the protection of at least one dose [51]. There are concerns that the plan is different from the efficacy trials and may lead to unchartered course of immune response. In addition, the approach could foster vaccine-resistant forms and increase the potential for escape mutants by having so many people with incomplete protection into a community swamped with SARS-CoV-2 infections. There is another concern, the vaccination programme is covering the elderly citizens first, in whom the immune system does not function so well, and some may inevitably contract the disease while waiting for their second dose of vaccine which may also erode confidence in the vaccines.

The moves are borne of a desire to begin vaccinating as many people as quickly as possible. The moves are also based on small slices of evidence mined from 'subsets of subsets' of participants in clinical trials, and on general principles of vaccinology rather than on actual research into the specific vaccines being used [52]. The US-CDC initially has opposed the British move, though it appears that with the scarcity of enough vaccine doses in the immediate future, the US may also resort to such proposition, which may be followed by other countries around the world. The move has been endorsed by the WHO [53].

Further, the use of a non-matching second dose of vaccine is also disturbing. While there is reason to believe boosting with a different type of vaccine might be useful in some cases, particularly if the first dose is a vaccine like the AstraZeneca vaccine that uses a harmless virus onto which genetic material from SARS-2 has been fused? But the approach has not been studied at all in clinical trials and may lead to unchartered course of immune response.

Conclusion: Dealing with the variants

The RBD and Non-RBD mutations: The RBD of the S protein in SARS-CoV-2 plays a crucial role in binding with the hACE2 receptors required for viral entry. There is an intricate ACE2 receptor recognition mechanism of the SARS-CoV-2

virus, which regulates its infectivity and pathogenesis [54]. The mutations involving the RBD, thus, are associated with altered infectivity and possibly disease severity. On the other hand, the mutations distal to the RBD also impact the transmissibility and the antibody can also target non-RBD regions. A mutation in non-RBD region may lead to polybasic cleavage sites enhancement, via electrostatic interactions and hydration and influence the RBD-ACE2 binding affinity [55].

It has been noted that certain mutations in the new variants affect genomic targets used by the PCR tests. This may affect the ability of some tests to detect the virus. However, because most PCR tests detect more than one gene target, there is a smaller chance of it causing a false negative result. Further, the mutations in various viral genes that help build glycan chains may influence the individual immune response, which is a more pressing concern relating to the antibody response to vaccines.

Recently, the actual sequence of the Pfizer mRNA vaccine was released [55]. The main difference in the vaccine code is that uracil has been replaced by 1-methyl-3'-pseudouridylyl, which is labelled as Ψ [57]. As such, the Ψ accepted as normal uracil by the immune systems for the translation, transcription, and replication reactions related to the cell functions. But the substitutions stabilize the S structure, and the deployment of special 5' and 3' untranslated regions before and after the main spike sequence. Whereas the Oxford-AstraZeneca vaccine contains the genetic material of the SARS-CoV-2 virus S protein. The new SARS-CoV-2 variants do not appear to affect the S protein much to prevent the immune response to the COVID-19 vaccines.

Immune escape variants and disease severity: Several genetic variants associated with immune escape have emerged in global populations. An immune escape variant of the SARS-CoV-2 is a mutation in the virus that allows it to evade the immune system. The CSIR Institute of Genomics and Integrative Biology (CSIR-IGIB) team has identified 120 'immune escape variants' in SARS-CoV-2 from across the globe [58]. In addition, the team has identified at least 19 genetic variants including the S:N440K variant, which have evolved to evade neutralising antibodies. Further, the team found 24 immune escape associated variants present in almost 70% of the viruses sequenced from Australia. The analysis also suggests that N501Y mutation was present in a total of 290 genomes, including genomes from Australia, South Africa, USA, Denmark, and Brazil.

It has been conjectured that the high rate of mutations in a virus may bring about its extinction. The speculation has been applied by Banerjee, et al. to SARS-CoV-2 with the hypothesis that the viral S glycoprotein is the key to viral binding, fusion, and cell entry leading to host cell infection, and increasing spike mutations may lower the viral fitness [59]. The authors used the genomic sequences of 630 Indian isolates retrieved from the GISAID database and found 41 and 22 mutations in the S1 and S2 subunits of the spike, respectively. Tracing the mutations showed that over two-thirds had lower spike-receptor stability than the ancestral strains, which implied that the rapid build-up of mutations in the recent variants leads to a loss of fitness compared to their ancestors. Thus, irreversible accumulation of harmful mutations causes a species to die out gradually. Similarly, accumulation of beneficial mutations can lead to enhanced viral fitness.

The number of infected cases, deaths, and mortality rates related to COVID-19 vary from country to country. The reasons of these regional differences are several [60]. There epidemiological variables related to the population factors such as relative ratio of different age groups, the individual host genetic factors, population density, and socioeconomic factors having a bearing on lifestyle, comorbid conditions, such as diabetes, cardiovascular, pulmonary diseases, and healthcare aspects including diagnostic workup. Further, apart from the known factors, with the evolving patterns of the pandemic, several novel epidemiological components including those related to the causative agent, SARS-CoV-2 virus such as mutations and new variants are being discovered and appear to influence the disease manifestations, its course, and later the convalescence phase as well.

There are several factors related to the virus, being discovered with the emerging patterns of COVID-19. In a study, Toyoshima, et al. identified a total of 1234 mutations by comparing with the reference SARS-CoV-2 sequence. Through a hierarchical clustering based on the mutant frequencies, the 28 countries were classified into three clusters showing different fatality rates of COVID-19. In the analysis, the authors correlated the ORF1ab 4715L and S protein 614G variants with fatality rates. They documented that BCG-vaccination status was significantly associated with the fatality rates as well as number of infected cases. In addition, the frequency of several HLA alleles, including HLA-A*11:01, was significantly associated with the fatality rates. These findings suggest that apart from SARS-CoV-2 mutations, BCG-vaccination status and a host genetic factor, HLA genotypes might affect the susceptibility to SARS-CoV-2 infection or severity of COVID-19.

The impact of SARS-CoV-2 variants: SARS-CoV-2, the virus that causes COVID-19, has had a major impact on human health globally by infecting large number of people, causing severe disease, and being associated long-term health sequelae resulting short-term as well as long-term morbidity and excess mortality, especially among older and vulnerable populations. It has led to interruption of routine healthcare services, trade, education and many other societal functions, and travel disruptions. Multiple SARS-CoV-2 variants are circulating globally, and several new variants have emerged lately in the last quarter of 2020.

Some of the potential consequences of emerging variants are the following:

- Rapid transmission - There is already evidence that one mutation, D614G, confers increased ability to spread more quickly than the wild-type SARS-CoV-2. Other variants, the British VUI-202012/01 variant and the South African 5O1Y.V2 Variant are also associated with enhanced infectivity.
- Disease severity - There is no evidence that the recently identified SARS-CoV-2 variants cause more severe disease than earlier ones.
- Ability to evade detection – These variants may evade certain diagnostic tests. However, most commercial PCR tests have multiple targets to detect the virus, so if the mutations impact some targets, the PCR will still work.
- Likelihood of decreased susceptibility to antiviral drugs such as remdesivir and favipiravir, and other therapeutic agents such as monoclonal antibodies.
- Likelihood of evading natural or vaccine-induced immunity. The multiple mutations in the S protein may confer ability to evade immunity induced by vaccines or by natural infection.

The impact of SARS-CoV-2 variants: The WHO is working with various countries to identify how current surveillance systems can be amplified and adapted to evaluate potential virus variations through ongoing systematic clinical and epidemiologic surveillance. In addition, it aims to establish of genetic sequencing capacity where possible and providing access to international sequencing services to send samples for sequencing and phylogenetic analysis.

As part of WHO's SARS-CoV-2 global laboratory network, the Virus Evolution Working Group (VEWG) - a specific working group on virus evolution was established in June 2020.

- The VEWG aims to –Strengthen mechanisms to identify and prioritize potentially relevant mutations,
- Early detection of relevant mutations and their potential impacts related to viral transmission and virulence, with relevance to available and prospective countermeasures including diagnostics and therapeutics,
- Evaluation of possible mitigation strategies to reduce the negative impact of mutations,
- Study of the impact of specific mutations, including in vitro and in vivo studies of variants in laboratory, and
- Sharing of full genome sequences and facilitating detailed analyses by scientists with expertise.

Simultaneously, the WHO is working to reduce transmission of SARS-CoV-2 by using established disease control measures as well as avoiding introductions to animal populations as part of the global strategy to reduce the occurrence of mutations that have potential negative public health implications [61]. The increased transmissibility has potential for higher case incidence, leading to increased COVID-19 related hospitalizations, and therefore, deaths. The WHO has warned that a more intensive public health response may be necessary to control variant transmission.

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Chapter 5: COVID-19 Pandemic, Recurrent Outbreaks and Prospects for Assimilation of hCoV-19 into the Human Genome

Background

The outbreaks and resurgence: The disease which reportedly began in the Chinese city Wuhan in November-December 2019, soon spread to various parts of the world, and was named and declared a pandemic disease by WHO. While the European countries were recovering from the epidemic, the disease took hold in the USA, the South American countries, Arabian countries, and South Asian countries, predominantly affecting Brazil, Peru, Iran, and India. Presently, many European countries are witnessing a resurgence and recurrent outbreaks of COVID-19.

Spread and evolving new insights: Whereas there is workplace-related infection rise as people are returning to their offices, in other places the outbreaks are related to the people crowding and meeting care-freely and trying to resort back to their earlier way of life. The reopening of the educational facilities across the continents may make matters worse.

Impact on health and healthcare: Most cases of COVID-19 infections go unnoticed and are followed by self-recovery. But what may appear good from the clinical perspective, appears to complicate epidemiological efforts to contain the outbreak. With the evolving information about the disease, there seem to be certain possible outcomes such as control and containment, or the persistence of the disease as global endemic accompanied with outbreaks and resurgent episodes.

Genetic factors linked to disease severity: With the COVID-19 pandemic, not all infected patients develop a severe respiratory illness. Further, there is a large variation in disease severity, which may be due to the genetic factors underlying the variable response to the virus. It is becoming clear that apart from the advanced age and pre-existing conditions, certain genetic constituent factors render some patients more vulnerable to the more severe forms of the diseases.

Integration of virus into human genome: A significant part of the human genome is derived from viruses especially the RNA viruses. In fact, about 8% of the human genome is made up of endogenous retroviruses (ERVs), which are viral gene sequences that have become a permanent part of the human lineage after they infected our ancient ancestors. With this background, a novel concept emerging that if COVID-19 persists for several generations, its genetic material is projected to be integrated or assimilated into human genome. The involved mechanisms are conceptualized through the transposons or transposable elements of the SARS-CoV-2.

The COVID-19 resurgence and recurrent outbreaks

The disease which reportedly began in Chinese city Wuhan in November-December 2019 manifesting as respiratory illness with upper respiratory symptoms and pneumonia-like illness, soon spread to various parts of the world, and was named and declared a pandemic disease by the World Health Organization. The disease soon spread to certain European countries. While these countries were recovering from the epidemic, the disease took hold in UK and USA, and the South American countries, Arabian countries, and South Asian countries, predominantly affecting Brazil, Peru, Iran, and India. Presently, most of the European countries are witnessing recurrent outbreaks and a resurgence of COVID-19, whereas the epidemic is not yet over in other countries. This calls for a need for balanced views about the preventive measures, renewed vigilance and new control strategies including the enforced lockdown measures specifically targeting clusters of outbreaks, which play a major role in the disease spread.

The rising number of cases in European countries, though, currently are not comparable to the peaks earlier during April- May 2020, is a matter of great concern. The inference from data from diagnostic testing appears to suggest that these countries might have relaxed the lockdown measures too early and too much [1]. In general, the people's willingness to stay alert and stick to new rules about social distancing and mask wearing appears to have vanished too, rather than becoming part of their behaviour pattern, leading to evolving new areas of fresh resurgence and outbreaks.

Evolving new insights into COVID-19 viral spread

Whereas there is workplace-related infection rise as people are returning to their offices, in other places the outbreaks are related to the younger people crowding, meeting and partying care-freely and other people trying to resort back to their earlier ways of life. Because most of the new outbreaks involve the cases in younger age groups, fewer of them die.

But it is only a matter of time before the elderly people are affected leading to higher mortality. Further, reopening the educational facilities across the continents may make matters worse.

The airborne transmission: The transmissibility of SARS-CoV-2 is high. The infected individuals release droplets and aerosols containing SARS-CoV-2 by coughing or sneezing. The virus-laden aerosols and droplets can lead to short-range airborne transmission (~ 6 ft). The transmission may occur through fomites also but appears to play a minor role.

Much depends on the size of the aerosols (< 10-μm diameter) and droplets (> 10 μm diameter) which can promote infection through direct inhalation and deposition on surfaces and subsequent hand-to-mouth/nose/eye transfer (Figure 1). The suspended airborne droplets appear to persist in the air for several minutes. Whereas the smaller aerosols can persist for longer durations (several minutes to hours). The characteristics of aerosols are dynamic, due to evaporative loss of water depending on ventilation, and ambient humidity and temperature levels. With decreasing size, their ability to disperse in air is enhanced, leading to their transmission extending beyond 6 ft from the point of release.

The aerosol size fractions may influence the deposition profile of SARS-CoV-2 in the lung [2]. Thus, whereas the larger aerosols (> 4 μm) are predominantly deposited in the upper and central airways (i.e., nasopharynx, tracheobronchial) and are subject to muco-ciliary clearance, the smaller aerosols (< 4 μm) get deposited deeper in the alveoli, having epithelial cells rich in ACE2, with enhanced transmission efficiency.

The emphasis on hand hygiene has been diluted, as it is becoming clear that contaminated surfaces may not play a large role. Similarly, the emphasis on banning outdoor activities is losing focus, as it is becoming established that the outdoor activities like jogging, outdoor hospitality, non-essential shopping, and public transportation are fine as long as people keep social distancing and wear face masks. Rather, the focus is on indoor activities which is the main culprit for the virus transmission.

The emphasis should now be on targeting outbreak clusters and super-spreading events. The studies indicate that 10% of patients cause 80% of all the infections, whereas most patients to the tune of 90% do not infect further [3]. The backward contact tracing is more useful than the forward tracing. Further, finding clusters help epidemiologists in understanding about the outbreaks. With more stress on the long-term care facilities and workplaces, the specifically targeted measures can help in preventing outbreaks, rather than general lockdown measures. These targeted versions include encouraging people to work from home and avoiding crowded places and banning meetings and gatherings.

Using the preventive measures and resources rationally, most of the countries are better prepared than before in the changed scenario. We are aware of the disease epidemiology, the virus transmission kinetics, and equipped with resources like PPE kits and masks. Further, the rational behaviour has emerged in place of irrational fears about the disease. The governmental organizations have developed machinery for contact tracing, surveillance and gathering data.

The impact of the disease on health and healthcare system

While most of COVID-19 infections go unnoticed and are followed by self-recovery. But what may appear good from

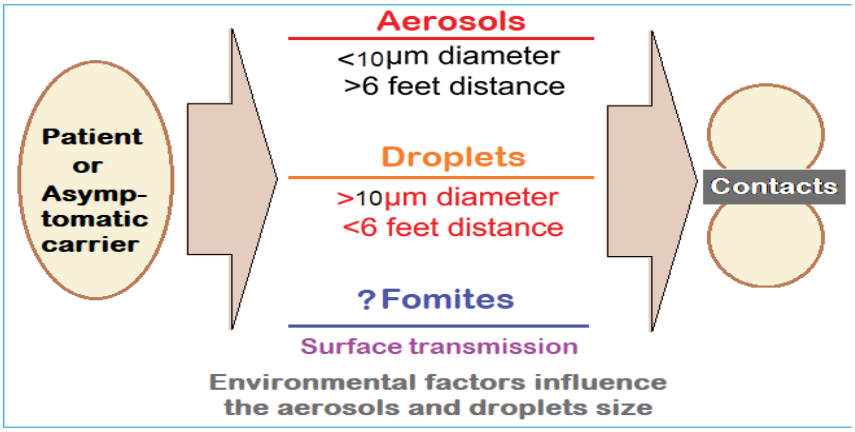

Figure 1: Transmission kinetics for SARS-CoV-2: Droplets and Aerosol Concepts.

the clinical perspective, vastly complicates epidemiological efforts to contain the disease outbreaks. With the evolving information about the COVID-19 so far, there appear to be following three possible outcomes.

Scenario 1: Control and Containment: The disease will persist with regional outbreaks and low endemicity. There will persist to occur periodic resurgence. The asymptomatic transmissions will continue to occur with occasional outbreaks and development of epicentres, requiring timely measures to curtail further spread.

Scenario 2: Persistence as global endemic: The overall mortality among known 2019-nCoV cases is about 2%. It appears that about 20% of infected people suffer severe disease [4]. Between these two groups are a multitude of people with milder forms of the disease, diagnosed or undiagnosed, who may or may not seek medical care. Many may have no symptoms at all. Thus, the diagnosed cases of the disease form the visible part of the largely hidden iceberg (Figure 2).

Scenario 3: If the disease goes on to persist and afflict the human populations over several generations, there are anticipated prospects of assimilation of hCoV-19 into Human Genome.

Genetic factors in clinical manifestations of COVID-19

With the COVID-19 pandemic, not all infected patients develop a severe respiratory illness; the reason for which is not apparent. Further, it appears that there is a large variation in disease severity, one component of which may be due to the genetic variability in the response to the virus [5]. The individual response to SARS-CoV-2 exposure and the vulnerability of individuals to the infection, and the clinical spectrum of COVID-19 are greatly variable (Figure 3). It is becoming clear that apart from the advanced age and pre-existing conditions, such as diabetes, cardiovascular, pulmonary, and renal diseases, certain genetic constituent factors render some patients more vulnerable to the more severe forms of the diseases, as is apparent from the rate of hospitalization of younger and apparently healthy individuals.

The host genetic factors have been linked to the variable clinical manifestations of the disease [6]. The clinical manifestations of COVID-19 patients have further been grouped based on (1) need for hospitalization, (2) need for oxygen supplementation, (3) progression to respiratory failure, or (4) mortality. From the clinical perspective, knowledge of host genetic factors could lead to improved care for patients with COVID-19. A model to understand human genomic variants linked to COVID-19 outcomes can be conceived as a continuum from ultrarare to common. Further, the genomic factors

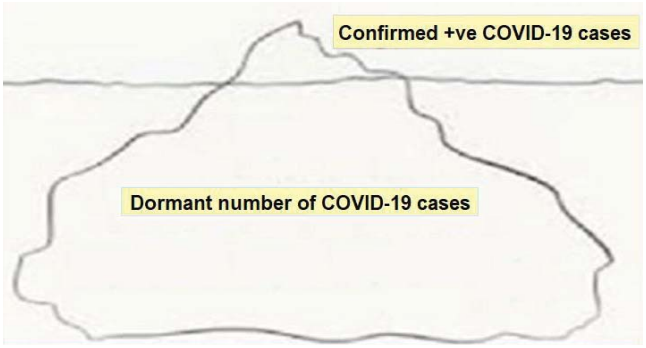

Figure 2: The COVID-19: Epidemiological Iceberg.

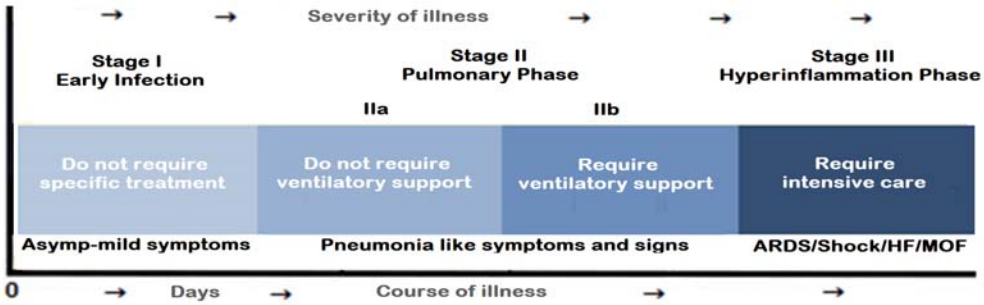

Figure 3: The clinical spectrum of COVID-19.

can be linked to variability in the protective immune response and have implications for vaccination strategies or could be used to optimally select patients for novel therapeutic treatments and trials.

The Genetic factors and Pathways related to COVID-19 involve -

1. The ACE2 gene encoding the ACE2 surface receptor associated with SARS-CoV-2 virus. The specific variants in the genes and pathways leads to difference in inter-individual COVID-19 susceptibility and response. The genetic polymorphisms in the ACE2 gene, which encodes the ACE2 receptors and allelic variants of the ACE2 may influence the virus binding subsequent to invasion of the cells.

2. Genes and pathways related to COVID-19 also include other viral receptor genes like related to TMPRSS2. The polymorphisms of cellular proteases, which facilitate the entry of SARS-CoV-2 into the cell, along with furin and TMPRSS2 - have been shown to exist. The TMPRSS2 variants and resulting expression may also influence COVID-19 severity, as well.

3. The course of the disease is also influenced by the inflammatory and immune response pathways such as IL-6 pathway, and genes involved in hypercoagulability and acute respiratory distress syndrome.

4. Other genes of interest include genes associated with ABO blood group [7]. A possible association between the genetic variability in histocompatibility complex (MHC) class I genes (human leukocyte antigen - HLA A, B, and C) and the susceptibility to SARS-CoV-2 and severity of COVID-19 has recently been suggested [8]. In Particular, the HLA-B*46:01 gene product is predicted to exhibit the lowest binding capacity to SARS-CoV-2 peptides and the individuals with this allele are more susceptible to COVID-19—due to reduced capacity for viral antigen presentation to immune cells. On the other hand, the HLA-B*15:03-encoded protein has the high capacity to present the conserved SARS-CoV-2 peptides that are shared among common human coronaviruses, leading the patients possessing this HLA genotype to more likely develop immunity.

Future prospects of COVID-19

Human genome and ERVs: A significant part of the human genome is derived from viruses [9]. In fact, the human genome is littered with various dormant viral genes [10]. About 8% of the human genome is made up of endogenous retroviruses (ERVs), which are viral gene sequences that have become permanently integrated parts of the human genome after infecting the human population groups during ancient times. The expression of these endogenous retroviruses has been implicated in diseases like autoimmune disorders and breast cancer. But they are also useful for human survival. For example, they play an important role as an interface between a pregnant mother and the fetus by regulating placental development and function.

Transposable elements or transposons: Viruses are ancient and vital simplest genetic constructions. They tend to be made of a protective shell, a protein called a polymerase, responsible for replicating the viral genome, and a sequence of nucleotides — either RNA or DNA — that encodes for the viral proteins. A virus may exist in purely genetic form, lacking a defined body. A stimulus disturbing its dormancy, lets it rebuild the physical body from a purely genetic form. The physical body bestows means and necessary tools to replicate. The disembodied viruses are called transposable elements, or transposons, which are mobile genetic elements also called jumping genes, and can move in and out of genomes. Transposons are present in all life forms. They are often the main components of the moderately repetitive DNA. In human beings more than 50% genome is composed of mobile elements (MEs) or Transposons [11].

Due to their past incremental accumulation and ongoing DNA transposition, MEs serve as a significant source for both inter- and intra-species genetic and phenotypic diversity in the primate and human evolution. The transposons can copy and paste themselves throughout genomes (Figure 4).

Some endogenous retroviruses (ERVs) are themselves transposons. As documented, nearly 8% of the human genome is made up of ERVs and nearly 50% of the human genome is made of transposons. The viruses through transposons are thought to play a major role in genetic change and influence the processes of evolution and speciation.

The proteins interacting with viruses: Over the course of the last several million years of evolution, the ancient human-beings likely to have been plagued by a multitude of viral epidemics. In this context, the host genomes offer an indirect way to detect the ancient epidemics [12]. These pathogens have shaped the host genomes by driving large

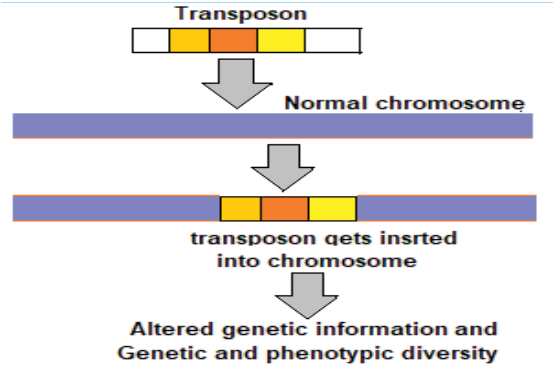

Figure 4: Transposons – the drivers of evolution.

numbers of adaptations involving various genes. A past epidemic can be detected through the enrichment in signals of adaptation at the host proteins that interact with viruses, called virus interacting proteins or VIPs [13].

By using the enrichment in signals of adaptation at about 4,500 host loci that interact with specific types of viruses, there has been documented that RNA viruses have driven large number of adaptive events across diverse human populations. Various types of viruses appear to have exerted different selective pressures during human evolution through the host VIPs. Further, the interactions with viruses account for approximately 30% of protein adaptations in the human genome, which have driven human evolution through various viral epidemics in past. Further, the analysis of VIPs suggests that the certain specific viruses may have indeed driven more epidemics than others in recent evolution.

The novel zoonotic RNA viruses: The RNA viruses are potentially the most important group involved in zoonotic disease transmission. They have higher probabilities to infect new host species because of their exceptionally shorter generation times and faster evolutionary rates. As documented and highlighted, the RNA viruses are the most common class of pathogens responsible for new human diseases, with a rate of 2 to 3 novel viruses being discovered each year [14]. RNA viruses show remarkable capabilities to adapt to new environments and confront the different selective pressures they encounter including the host's immune system and defense mechanisms.

The recent emergence of interspecies-transmitted RNA viruses, such as Chikungunya (CHIKV) and Zika (ZIKV) viruses, represent new global pandemics. Other zoonotic, highly communicable RNA viruses during recent years include Lassa fever, Ebolavirus, severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and Influenza A virus (IAV) [15].

The proofreading proteins in CoVs: The peculiar rate of adaptive evolution of the RNA viruses arises from their exceptionally high mutation and substitution rates, with the RNA viruses showing greater substitution rates than DNA viruses. Further, most RNA viruses lack the proofreading ability of RNA polymerase and Reverse transcriptase. A fallout of this is that in long run they lose infectivity as the number of non-viable viruses outnumber those viable ones. Whereas in the case of replicative DNA polymerases of DNA viruses and cellular organisms, an exonuclease corrects the possible nucleotide misincorporations during the genome replication.

The coronaviruses (CoVs), like the DNA viruses, have the potential proofreading functions as the nsp14 protein acts as a 3′-5′ exoribonuclease on both single-stranded and double-stranded RNA during the viral replication cycle [16]. Through this proof-reading function, the CoVs appear to have overcome the limitation, which in most RNA viruses, during process of replication, lead to the non-viable virions rapidly outnumbering the viable ones, leading to a loss of fitness and/or viral extinction. In addition, the CoVs, like other RNA viruses can resort to RNA viral evolution through recombination (synthesis of chimeric RNA molecules from two different progeny genomes) and reassortment (the packaging within a single virion of genomic segments from different progeny viruses) [17].

Conclusion: projected SARS-CoV-2

Integration into human genome

Various salient features in structure and physiology of the SARS-CoV-2 make it a highly pathogenic RNA virus, which is

likely to persist as infecting agent and can potentially afflict the human populations for innumerable years if not for many generations. With this background, an emerging novel concept holds that if COVID-19 persists for several generations, its genetic material is projected to be integrated or assimilated into human genome. The involved mechanisms have been conceptualized through the transposons or transposable elements of the SARS-CoV-2.

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Chapter 6: Living with 'Long COVID-19': The Long-term Complications and Sequelae

Background

Introduction - The perennial pandemic: It is being increasingly realized that the COVID-19 may have become the new reality associated with human existence world over and the mankind may have to live with it for years or even decades. Further, the grievous nature of the disease is evolving further with the genomic changes in the virus in form of mutations and evolution of variants, with enhanced infectivity and probably virulence. There are serious challenges posed by the SARS-CoV-2 virus and COVID-19 as the disease.

COVID-19 as acute and chronic disease: On exposure to the SARS-CoV-2 virus, not all patients develop a disease. Further, for those who develop the disease, there is a large variation in disease severity. The known factors including the constituent factors and several still unknown factors may influence the disease manifestations, its course, and later the convalescent phase as well. In fact, there is a growing evidence of persisting multisystem effects of COVID-19, indicating substantial continuing morbidity after resolution of the infection.

The 'long COVID-19' or 'long haulers': The patients who continue to suffer with persisting symptoms have been described as long haulers and the clinical condition has been called post-COVID-19 or 'long COVID-19'. The diagnosis should be entertained if various symptoms and signs linger well beyond the period of convalescence in COVID-19. With the chronicity, there occur inflammatory changes and damage in lungs, heart, immune system, brain, the vasculature, and other organs, and the extent of organ damage determines the long-term effects.

Management of 'Long COVID' syndrome: The 'long COVID' syndrome has multi-system involvement, variable presentation and unpredictable course depending on known factors such as race, age, sex, and comorbidities, and certain unknown factors. Following clinical and investigational assessment, the patients should be managed as per clinical manifestations, extent of organ damage and associated complications. The findings from various studies indicate that preventing further organ damage in 'long COVID' is crucial.

The Long COVID's prognostic challenges: As apparent, the 'long COVID' afflictions are more common than realised earlier. The symptoms can escalate in patients with co-morbid conditions. The persistent symptoms among COVID-19 survivors pose new challenges to the healthcare providers and may be suitably managed with a combination of pharmacological and non-pharmacological treatments, and holistic healthcare. In addition, by reducing the inflammation and continuing damage in various organs, the prognosis can be improved.

Introduction: The perennial pandemic

It is being increasingly realized that the COVID-19 may have become the new reality associated with human existence world over. The mankind may have to live with COVID-19 for years or even decades [1]. Following infection, the C19 virus becomes an intracellular entity that has penetrated the cell walls. It may not be possible to destroy the intracellular virions. Further, while we continue to explore the agent factors, disease transmission dynamics, pathogenesis and clinical spectrum of the disease, and therapeutic modalities, the grievous nature of the disease is evolving further with the genomic changes in the virus and pathophysiological alterations and clinical manifestation. The future of the disease, of course, is stated to depend on various known and unknown factors, many of them may not be modifiable. Let us learn to live with this reality.

Apart from being a pandemic, COVID-19 has become a perennial disease. There are serious challenges posed by SARS-CoV-2 virus and COVID-19 as the disease that are further increased by mutations and evolution of variants with enhanced infectivity and probably virulence. The significance of viral mutations needs to be highlighted and investigated further, which may help in designing therapeutics to combat the disease as well as developing effective vaccines for its prophylaxis. In addition, the fields of improving the immune response to the infection and vaccine shots and the immunity in general are to be explored and harnessed. Looking forward, let us hope that the today's unmet challenges are resolved in near future [2].

COVID-19 as acute and chronic disease

Acute COVID-19: The clinical spectrum: Being exposed to the SARS-CoV-2 virus, not all infected patients develop a

disease. It may be remembered a significant number of those get infected with the SARS-CoV-2 virus are asymptomatic: In fact, a recent systematic review has documented that at least one-third of SARS-CoV-2 infections occur in people who never develop symptoms, highlighting the substantial prevalence of asymptomatic infections [3]. The study included serologic surveys from more than 365,000 people in England and more than 61,000 in Spain. When analysed, a similar proportion of asymptomatic cases 32.4% in England and 33% in Spain was noted.

Further, for those who develop the disease, there is a large variation in disease severity, one component of which may be due to the genetic variability in the response to the virus [4]. The individual response following the SARS-CoV-2 exposure and the vulnerability of individuals to infection, and the clinical spectrum of COVID-19 is greatly variable (Figure 1).

The clinical manifestations of COVID-19 patients have been grouped based on the need for hospitalization, need for oxygen supplementation, and progression to respiratory failure, or mortality. Presently, considering the post-illness symptoms and manifestations, the resolution phase can be added to this.

Factors influencing the disease course: Further, it is becoming clear that apart from the advanced age and pre-existing conditions, such as diabetes, cardiovascular, pulmonary, and renal diseases, certain constituent factors render some patients more vulnerable to the more severe forms of the diseases, as is apparent from the rate of hospitalization of younger and apparently healthy individuals. The known factors including the constituent factors and several still unknown factors may influence the disease manifestations, its course, and later the convalescent phase as well. From the clinical perspective, knowledge of host constituent factors, including the genetic variations, could lead to improved care for patients with COVID-19 during the acute phase of the disease as well as during the resolution phase encompassing convalescence and 'long COVID' phase. The host genetic factors have been linked to the variable clinical manifestations of the disease following exposure to the virus at the individual level as well as in various population groups [5]. In fact, a model to understand several related factors such as the age, associated co-morbidities and genetic factors including human genomic variants linked to COVID-19 outcomes can be conceived as a continuum from ultrarare to common (Figure 2).

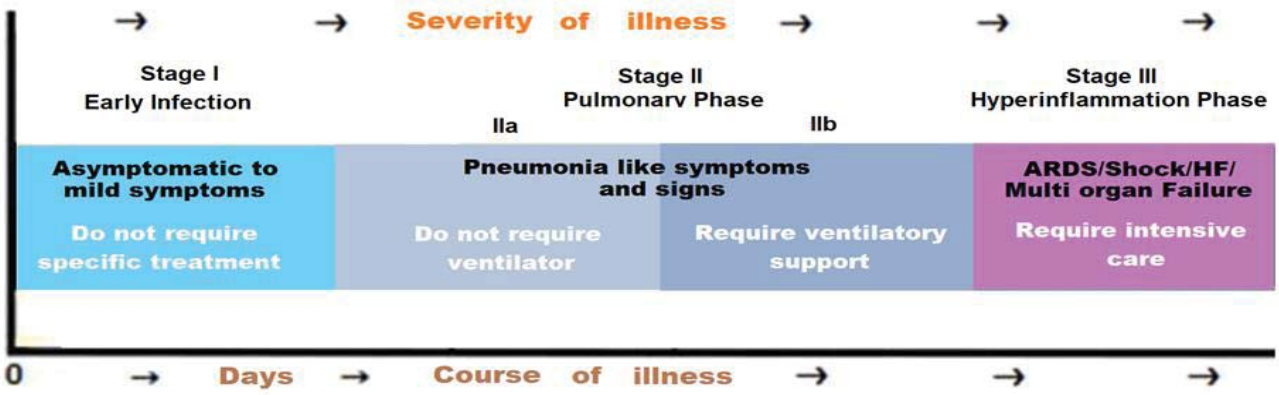

Figure 1: The clinical spectrum of COVID-19.

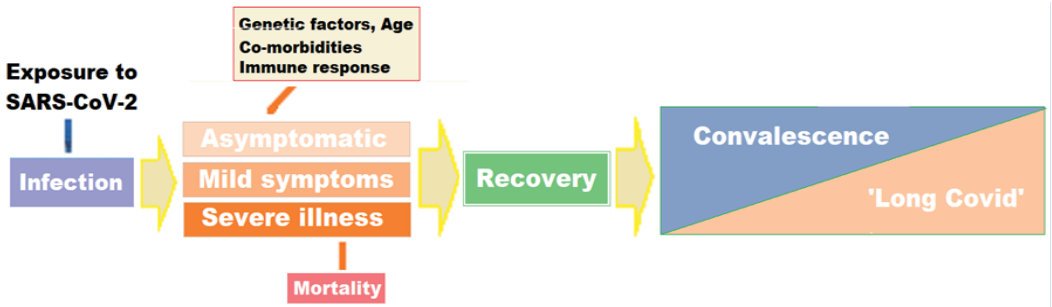

Figure 2: The factors related to the disease - clinical course, convalescence, and 'long-COVID-19.

The genetic factors and pathways related to COVID-19 involve the ACE2 gene encoding the ACE2 surface receptor associated with SARS-CoV-2 virus. The specific variants in the genes and pathways leads to inter-individual COVID-19 susceptibility and response. The genetic polymorphisms in the ACE2 gene, which encodes the ACE2 receptors and allelic variants of the ACE2 may influence the virus binding subsequent invasion of the cell. In addition, the genetic factors and pathways also include the polymorphisms of cellular proteases, which facilitate the entry of SARS-CoV-2 into the cell, along with furin and TMPRSS2. The TMPRSS2 variants and resulting expression may also influence COVID-19 severity, as well. Other genes of interest include genes associated with ABO blood group [6].

There is a possible association between the genetic variability in histocompatibility complex (MHC) class I genes (human leukocyte antigen - HLA A, B, and C) and the susceptibility to the virus and disease severity [7]. In particular, the HLA-B*46:01 gene product exhibits the low binding capacity to SARS-CoV-2 peptides resulting in the individuals with this allele being more susceptible to COVID-19 due to reduced capacity for viral antigen presentation to immune cells. On the other hand, the HLA-B*15:03-encoded protein has a high binding capacity to SARS-CoV-2 peptides and present to immune cells leading the patients with this HLA genotype to develop better immunity. The human genomic factors can also be linked to variability in the protective immune response through the inflammatory and immune response pathways such as IL-6 pathway, and genes involved in hypercoagulability and acute respiratory distress syndrome. The variable immune response has implications for vaccination strategies and for optimally selecting patients for novel therapeutic treatments and trials.

To track the acute as well as long-term impact of the disease on health, the project called the COVID Human Genetic Effort, aims to find genetic variants that compromise the immune systems and making certain individuals more vulnerable to the risk of developing COVID-19. In addition, it aims to document the genetic variations which make some individuals resistant to the SARS-CoV2 infection [8]. In nutshell, the project aims to discover – (1) Monogenic inborn errors of immunity (IEI), rare or common, underlying severe forms of COVID-19 in previously healthy individuals, and (2) Monogenic variations which make certain individuals resistant to the SARS-CoV2 infection despite repeated exposure, and (3) decipher the molecular, cellular, and immunological mechanisms by which they cause resistance to viral infection or predisposition to a severe form of the disease.

COVID-19 delayed and long-term effects: There is a growing evidence of persisting multisystem effects of COVID-19, indicating substantial continuing morbidity after resolution of the infection [9]. While most people recover quickly and completely from the virus, persistent and troubling symptoms are frequently seen and reports from cohort studies suggest that one in three people have not fully recovered several weeks after initial illness and a smaller but still substantial proportion have symptoms and difficulties that persist for months [10]. Further, the long-term effects of COVID-19 are seen in the younger population also, though the risk of 'long COVID' increases with age. People who suffer with severe form of the disease, experience long-term inflammation and damage in lungs, heart, immune system, brain, the vasculature, and other organs. These long-term effects can last for months and years. The 'long COVID' is not contagious and result of the body's response to the virus infection continuing beyond the initial illness. Thus, the unmitigated COVID-19 infection has impact on the health of all groups and the strategies for prevention and suppression of COVID-19 should focus the older people or those with comorbidities, but also the younger population.

Most COVID-19 patients recover within a few weeks without significant complications. But some patients, even those who had mild versions of the disease, including the younger people afflicted with mild or asymptomatic disease and those who did not suffer with serious disease or require hospitalization, may continue to experience symptoms after their initial recovery [11]. These patients have been described as long haulers and the clinical condition due to persisting or continuing symptoms has been called post-COVID-19 syndrome or 'long COVID-19'. The clinical condition encompasses a delayed convalescence or recovery, persistence of symptoms, and emergence of symptoms related to the organs involved and damaged, and incapacitating complications and sequelae (Figure 3).

Often the symptoms may go un-noticed, as they are vague and nonspecific, but persist making the patients to suffer with the COVID-19-related ordeals. Further, of various the facets of the disease, this one may ultimately prove to be the most difficult to deal with. The long-haul COVID patients carry their symptoms well beyond the normal course of recovery lasting for weeks and months or longer. These symptoms are often varied and relatively common and may defy a COVID-related diagnosis. Several patients who are expected to recover, continue to suffer for a variable period of weeks and months with various general symptoms such as fatigue, dizziness, memory lapses and other cognitive issues,

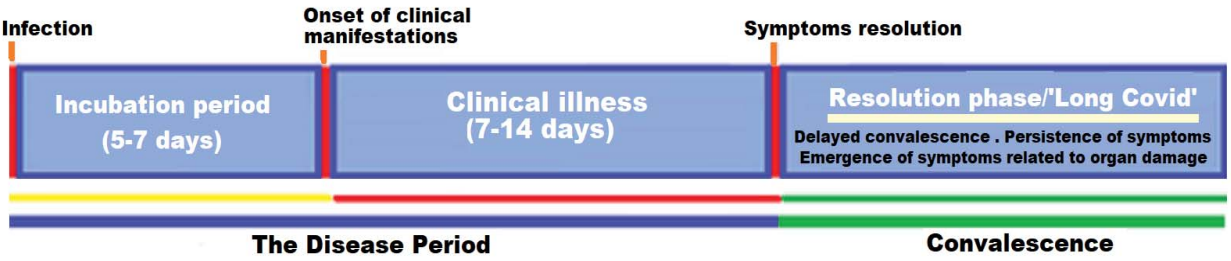

Figure 3: The abnormal convalescence or resolution phase following Covid-19 illness: delayed convalescence, persistence of symptoms, and emergence of symptoms related to organs affected, and prolonged recovery and incapacitating complications and sequelae.

digestive problems, erratic heart rates, headaches, fluctuating blood pressure, and muscular and joint pains, which are often considered by the patient himself and family members as related to the weakness developed following the disease.

The older people and those with serious co-morbid medical conditions are the most likely to experience lingering COVID-19 symptoms, but even young, otherwise healthy people can continue to suffer and feel unwell for weeks to months after infection. These patients are reporting weeks and months-long symptoms that affect various organs. Given the multitude of COVID-19 cases worldwide, the prevalence of 'long COVID' is expected to be substantial and likely to increase with the recurrent outbreaks of the disease. It is feared that the 'long COVID' with debilitating and prolonged illness may have profound impact on health of people, their social life and livelihoods, and the economy.

The 'long COVID-19' or 'long haulers'

The presentation of 'long COVID' symptoms: The occurrence of persistence or appearance symptoms related to 'Long-COVID' are being increasingly reported. Over the past few months evidence has mounted about the serious long-term effects of COVID-19 and it is estimated that we probably have way more than five million people with long COVID. The worldwide percentages of infection suggest that many of those people are living and suffering in the U.S. The 'long COVID is neither well-defined nor well understood, partly because the related research is still in its infancy. The syndrome has vastly emerged from self-reporting and appears to be a real clinical entity with the chronic health manifestations. The symptoms persist or develop outside the initial viral infection, and the duration and pathogenesis are unknown. Late sequelae have been described even in young, healthy people who had mild initial infection, and there is often a relapsing and remitting pattern. There are vague to severe incapacitating symptoms.

The syndrome has been documented to affect a significant number of individuals. Further, the post-COVID symptoms tend to be more common, severe, and longer-lasting than other viral illnesses, such as influenza. The cause of 'long COVID' is not known. It may be due to an immune-inflammatory response gone berserk or related to persistent viraemia due to weak or absent antibody response and the ongoing viral activity. Other factors like and psychological factors such as post-traumatic stress and deconditioning may be involved leading to neuropsychiatric sequelae. The long term respiratory, cardiovascular, and musculoskeletal injuries have also been described for SARS and MERS, and the post-acute COVID-19 is likely to have similar pathophysiology. The etiology is likely to be multifactorial and may involve overzealous immune responses, cardiopulmonary or systemic inflammation, vascular inflammation and coagulation disorders, and a direct cellular damage from viral replication during acute illness. Further, there is little known about the prevalence, risk factors, or possibility to predict about the protracted course early in the course of the disease. In general, the 'Long COVID' is characterised by symptoms of fatigue, headache, dyspnoea, and anosmia and likely to increase with age, higher BMI, and female sex. Further, as documented experiencing more than five symptoms during the first week of illness is associated with 'Long-COVID'.

In a study with online survey data involving over 4,000 COVID-19 patients, about 13.3% of all ages suffered with the symptoms lasting > 28 days, whereas 10% of those aged 18-49 years had the related symptoms 4 weeks after acquiring the infection. Further 4.5% of all ages suffered with the symptoms for more than 8 weeks, and 2.3% of all ages for more than 12 weeks. The study was conducted by health-science firm Zoe Global Limited in conjunction with Biomedical Research Centre based at GSTT NHS Foundation Trust and supported by the UK Research and Innovation [12]. The analysis and inference derived from similar studies could be used to identifying individuals with 'Long-COVID' may help to reduce long-term complications and sequelae and planning health education, guidance, and rehabilitation services [13].

Diagnosis and manifestations of 'Long COVID': As a matter of fact, medical advice should be routinely sought for all the patients having delayed recovery and persistence or emergence of symptoms. There is a multitude of adverse physical and mental health effects due to COVID-19. The evidence from other coronavirus infections such as the severe acute respiratory syndrome (SARS) and MERS epidemic suggests that these COVID-19 afflictions may last from months to years. This being the other side of the disease, a prolonged medical follow-up of COVID-19 patients is essential. According to a study published in the Lancet, which included 1,733 people tested positive for COVID-19 and followed for 4 months, found that more than 75% of the people who were hospitalized for COVID-19, suffer with at least one symptom 6 months after recovery. Further, it was noted that about 76% of them experienced lingering symptoms of COVID-19 long after being cured of the illness [14].

From the clinical perspective, the COVID-19 incubation period and clinical phase involves 3 weeks, the post-acute COVID can be described as the illness extending beyond three weeks from the onset of first symptoms, and the chronic COVID-19 as extending beyond 12 weeks. Around 10% of patients who have tested positive for SARS-CoV-2 virus remain unwell beyond three weeks, and a smaller proportion for further period. The diagnosis of the 'Long COVID' or 'Long haulers" should be entertained for various symptoms and signs linger well beyond the period of convalescence in COVID-19.

- The most common of persisting signs and symptoms of post-COVID-19 illness include:Extreme tiredness (fatigue), giddiness, paraesthesia, and joint and muscle pains
- Chest pain and tightness, palpitations, shortness of breath and Cough
- Persistence of Loss of smell or taste
- Gastrointestinal upsets such as anorexia, nausea, vomiting, diarrhoea, and stomach aches
- Headache and neurocognitive difficulties related to memory and concentration ('brain fog')
- Mental health conditions such as anxiety, insomnia, and depression, and post-traumatic stress syndrome
- Metabolic disruptions such as altered thyroid function, poor control of diabetes, etc.
- Seizures, facial paralysis, impaired vision and hearing, and tinnitus and earaches
- Skin rash like vesicular, maculopapular, urticarial, or chilblain-like lesions and hair loss.

The post-exercise malaise following physical activity and chronic fatigue are the commonest symptoms witnessed in 'long COVID' during a follow up of 6 months. It has been experienced by a substantial number of patients that the relapses are triggered by stress or exercise and they are unable to work at full capacity (about 45% required a reduced work schedule and about 22% were not working at all) prior to the infection. Another most insidious long-term effect of COVID-19 is cognitive dysfunction (or 'brain fog'). Following the infection, a significant number of patients report crippling exhaustion and malaise and find ordinary activities like getting out of bed or going to work tiresome. In a study of 143 COVID-19 patients discharged from a hospital in Rome, about 53% complained of fatigue and 43% of shortness of breath [15].

Further, the patients with severe COVID-19 disease commonly experience sequelae affecting their respiratory status, physical health, and mental health for at least several weeks after hospital discharge. A study of patients in China showed that about 25% of them had abnormal lung function after 3 months, and that 16% were still fatigued [14]. The symptoms resemble chronic fatigue syndrome, also known as myalgic encephalomyelitis (ME). Since there being no biomarkers, diagnosis is based on symptoms. Further, the chronic fatigue is not limited to severe cases and common in even those who had mild symptoms. The persistent low-level inflammation triggered by infection appears to be the cause partly. The treatment is only imperative.

The study by Davis, et al. surveyed more than 3,700 people from 56 countries who had contracted COVID-19 between December 2019 and May 2020 [16]. There were recorded 205 symptoms across 10 organ systems and traced 66 symptoms over 7 months. Over 65% of respondents experienced symptoms for at least 6 months. The most often reported symptoms were fatigue and post-exercise malaise, in addition to vague neurological sensations, headaches,

memory issues, muscle aches, insomnia, palpitations, shortness of breath, dizziness, gait imbalance and speech disorders. In general, the long-term neurological symptoms were common even among patients with less severe disease. The less common manifestations of 'long COVID' reported are seizures, facial paralysis, impaired vision and hearing, prolonged loss of taste and smell, and some form of allergies [17,18].

In another recent study, Carvalho-Schneider, et al. followed-up of 150 adults with only mild to moderate COVID cases for two-month and found that two thirds of them were still experiencing symptoms, most commonly shortness of breath, loss of smell and taste, and/or asthenia and fatigue [19]. Another study by Italian researchers, covering 143 COVID patients who had been discharged from the hospital, found that only about one in eight was completely free of symptoms 60 days from the beginnings of the illness [20]. The King's College London study, one of the largest surveys so far, reported that around 10% of patients had persistent symptoms for one month, with 1.5% to 2% having sustained symptoms at three months. Further, the study documented that long COVID was twice as common in women as men, and the older people, and those with more than five symptoms during their first week of illness were more likely to develop 'long COVID' [12].

The organ involvement in 'Long COVID': COVID-19 as a disease primarily affects the lungs, but the virus can attack and injure other organs and cause some a multitude of symptoms. COVID-19 often strikes the lungs first, as the cells in upper and lower respiratory tracts richly harbour the ACE2 receptor which is the major target of SARS-oV-2 virus. In addition, the infection can harm the immune system, which pervades the whole body. Some people who have recovered from COVID-19 could be left with a weakened immune system. For a long time, they are likely to be immunosuppressed and vulnerable to other infections.

In general, the extent of organ damage determines the long-term effects. The organs and systems affected by COVID-19 include:

Lungs - The lung involvement in COVID-19 cause alveolar damage depending on the disease severity [21]. The alveolar damage is followed by pulmonary fibrosis resulting in long-term respiratory symptoms [22]. In the light of the pathophysiology of COVID-19, as the disease begins and primarily affects the respiratory system, the lungs are the obvious place to check for long-term damage [23].

The lung damage associated with the 'long COVID' on the chest scans reveals that even in the early weeks following recovery from COVID-19 infection, some patients have distinct signs of alveolar tissue damage leading to scar and fibrosis. As noted by Gholamrezanezhad, et al, in majority of the confirmed COVID-19 patients, computed tomography (CT) examinations yield a typical pattern with the sensitivity of 97% [24]. On follow up, in more than one-third of patients after one month, the tissue damage leads to visible scars and these patients representing with intermediate-term lung damage are likely to experience lasting respiratory symptoms. In an Italian study, a large proportion of patients (over 70%) with COVID-19 presented during follow up with symptoms including cough, fever, dyspnoea, musculoskeletal symptoms (myalgia, joint pain, fatigue), gastrointestinal symptoms, and anosmia/dysgeusia [9].

The persistent shortness of breath and not being able to carry out the usual exertional activities like climb up a few flights of stairs, without getting breathlessness are common symptoms seen in 'long COVID' patients. Various small studies have correlated the lung findings like fibrosis with the persistent lung symptoms. A retrospective multicenter study involving 55 recovered noncritical patients documented that over 60% patients had persistent symptoms three months following discharge, whereas over 70% had abnormal findings on lung CT scans and about 25% had demonstrable significant abnormalities in pulmonary function tests [25].

It has been found that the lower lobes of the lungs are the most frequently affected. The CT scans during the acute infection commonly show opaque patches indicating inflammation, which usually reduce in most cases after two weeks. An Austrian study also confirmed that lung damage seen on scans lessened with time: 88% of participants had visible damage 6 weeks after being discharged from hospital and after 12 weeks only 56% showed lung damage [26]. It has been noted in a prospective follow-up of patients infected with the coronavirus that COVID-19 patients may suffer long-term lung and heart damage but, for a significant proportion, this tends to improve over time [27]. On the other hand, though the lung scans can improve with time, many patients continue to suffer with lasting symptoms which may take a long time to resolve, as highlighted in post-discharge patients 70% of whom continued to suffer with shortness of breath and 13.5% were still using oxygen at home [28]. The past evidence from people infected with SARS and MERS also suggests

that the lung injury and symptoms may linger for some. This is also suggested by a study published in February 2020 highlighting the long-term lung harm in SARS patients due to SARS-CoV-1, in which even after 15 years (between 2003 and 2018) 4.6% of SARS patients had visible lesions on their lung scans, and 38% had reduced diffusion capacity [29].

Heart – Various clinical studies have shown that COVID-19 patients may suffer with complications like myocarditis and cardiomyopathy, abnormal heart rhythms and other cardiac sequelae weeks and months after contracting the infection [30]. The myocardial damage increases the risk of heart failure and other related complications [31]. The exact prevalence of cardiovascular disease (CVD) in COVID-19 patients is not known, but pre-existing CVD may be associated with a more severe COVID-19 infection [32].

It appears that about 20% of patients admitted with COVID-19 have clinically significant cardiac involvement. Cardiopulmonary complications include myocarditis, pericarditis, myocardial infarction, dysrhythmias, and pulmonary embolus; they may present several weeks after acute COVID-19. They are commoner in patients with pre-existing cardiovascular disease, but they have also been described in young, previously active patients. The occult involvement is common and add to post-COVID complications [33].

Various related studies appear to help explain the cause of shortness of breath, palpitations, and other cardiac symptoms in 'long COVID' patients. The imaging studies may indicate damage to myocardium weeks and months following recovery, even in those who had only mild COVID-19 symptoms. In a non–peer reviewed study involving 139 health care workers following recovery from COVID-19, 37% of them were found to have developed myocarditis and myopericarditis on their scans about 10 weeks after initial infection, but only half of them had showed related symptoms [34].

Brain – The 'long COVID' is commonly associated with mild neurologic symptoms such as 'brain fog', dizziness, headache, insomnia, loss of smell or taste, etc. [35]. On the serious side, in any age group, COVID-19 can cause ischemic strokes, cranial neuropathies and Guillain-Barre syndrome during the acute phase as well as during the chronic phase. Carlos del Rio, et al. have described encephalitis due to the virus and occurrence of 'brain fog' and seizures several months following the initial infection [11]. Further, COVID-19 increases the risk of neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease in long term.

It has been found that the serious neurologic manifestations are more likely to occur in patients who have suffered with severe COVID-19 disease, are older and have co-morbidities [36]. In addition, these patients are more likely to present with myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) like other viral infections such as SARS, MERS, and HIV.

In addition, they are also more likely to suffer with neurological complications like cognitive difficulties, including confusion and memory loss, persisting for a longer duration.

It appears that the virus may not infect the brain and the symptoms are a secondary consequence of cerebral inflammation [37]. The various mental health related outcomes such as anxiety, hopelessness, 'brain fog', depression and post-traumatic stress disorder are more common in patients with serious disease and may have a link with cerebral inflammation. Further research and long-term studies in due course may provide better understanding of the neurological and psychological manifestations of COVID-19.

Blood vessels - COVID-19 affects small blood vessels as well as large vessels leading to vasculopathy and coagulopathy. The involvement of major vessels can result in acute coronary and cerebrovascular events, whereas the involvement of small blood vessels, arterioles, and capillaries in the heart lead to myocarditis and cardiomyopathy, and acute renal injury, acute injuries in other organs.

In addition, the immune system becomes overactive and triggers harmful widespread inflammation and organ damage. The over-reactive immune system through inflammation and tissue damage leads to cardiovascular manifestations such as cardiomyopathy and pulmonary thrombosis in almost one-third of patients during the acute phase of COVID-19, affecting the ejection fraction of heart. In addition, the virus can infect the endothelial cells in blood vessels of various organs [38]. The cardiovascular system affliction may linger on for a prolonged time. The patients with COVID-19 pneumonia are at increased risk of long-term cardiovascular manifestations [39].

Further, various long-term COVID-19 related effects are still unknown and may become apparent only later. There are concerns that the pandemic will lead to a significant surge of people battling with lasting symptoms and disabilities

resulting due to the infecting virus and subsequent inflammatory process. In addition, some of the organ injury is likely to be a side effect of intensive treatments such as intubation, ventilatory therapy related and therapeutic agents used during hospitalization.

Management of 'Long COVID' syndrome

There is a healthcare continuum for management of 'long COVID' encompassing diagnostic workup, clinical follow up, investigational workup, followed by assessment of organ damage and treatment which involves pharmacological as well as non-pharmacological treatment, and holistic care (Figure 4).

The findings from various clinical and follow up studies of those infected with SARS-CoV-2 are crucial in preventing further organ damage in 'long COVID'. They are equally important for developing clinical guidelines for the care of survivors of COVID-19 afflicted with persistent and incapacitating symptoms. Presently, the physicians and medical communities are getting aware of long-hauler syndrome and the need of establishing post-COVID clinics with multidisciplinary and integrated approach is being realised. Further, the Neuro COVID-19 and Pulmo COVID-19 Clinics could be novel concepts for dealing with the 'long COVID' patients and offering them specialized care and follow up.

Approximately 10% of people experience prolonged illness after acute mild or severe COVID-19. Many such patients recover spontaneously with holistic support, rest, symptomatic treatment, and gradual increase in activity. Further specialised treatment is required for new, persistent, progressive, and newly emergent respiratory, cardiac, or neurological symptoms. As such the post-acute COVID-19 or 'long COVID' seems to be a multisystem disease and its management is largely based on limited clinical studies and requires a whole-patient perspective [40].

General treatment guidelines:

Respiratory symptoms: A degree of breathlessness is common during acute COVID-19 and convalescence. There could be worsening breathlessness and severe breathlessness may require hospitalization. In general, the breathlessness tends to improve with breathing exercises and guided pulmonary rehabilitation.

Chest pain: Non-specific chest pain is common in post-acute COVID-19. It could be musculoskeletal, unexplained non-specific chest pain, or due to a cardiovascular condition.

Thromboembolism: COVID-19 is an inflammatory and hypercoagulable state, with an increased risk of thromboembolic events. The hospitalized patients, in general, receive prophylactic anticoagulation but the recommendations for anticoagulant therapy after discharge vary. The high-risk patients are typically discharged with 10 days of extended thromboprophylaxis. Investigations and monitoring should follow during follow up.

Ventricular dysfunction and cardiopulmonary complications: Left ventricular systolic dysfunction and heart failure can occur during post-COVID period. Intense cardiovascular exercise must be avoided for three months in all patients with myocarditis or pericarditis. During the follow up they are to be assessed for functional status, absence of dysrhythmias, and left ventricular systolic function.

Mental health and wellbeing: Anxiety, stress, and symptoms related to social isolation are common. The post-COVID period is often associated with low mood, hopelessness, and insomnia. Post-traumatic stress disorder may occur and common in healthcare workers.

The older patient: In general, COVID-19 tends to affect older patients more severely. Post-COVID-19 chronic pain

Figure 4: The 'long Covid' care and management continuum – Diagnosis, Clinical follow up, Investigations, Assessment of organ damage, and Supportive treatment.

and other general symptoms are frequent. The elderly patients, in addition, are more prone to risk of sarcopenia and malnutrition. Depression and delirium in elderly may mask neurodegenerative disorders like Alzheimer's disease.

Investigations during 'Long COVID' follow up: Blood tests - Anaemia should be excluded. Lymphopenia is a feature of severe, acute COVID-19 illness, whereas leucocytosis may denote infection or inflammatory response.

The elevated biomarkers may include C reactive protein, natriuretic peptides in heart failure, ferritin indicative of inflammation and continuing prothrombotic state, troponin suggestive of acute coronary syndrome or myocarditis and D-dimer indicating thromboembolic disease.

A chest X-ray at 12 weeks and referral for new, persistent, or progressive symptoms. Cardiac echo, CT scan chest or MR scan may be required.

Further studies and research are likely to refine the indications and interpretation of diagnostic and monitoring tests in follow-up of 'long COVID'.

Supportive treatment and rehabilitation of 'Long COVID': The 'long COVID' syndrome has multi-system involvement and unpredictable course with variable presentation depending on known unmodifiable factors such as race, age and sex, and modifiable factors like comorbidities and lifestyle, and certain unknown factors. Following clinical and investigational assessment, the patients should be managed according to their clinical manifestations, extent of organ damage and associated complications.

***Planning careful resumption of physical activity*:** As highlighted earlier, apart from the severe cases, even those who suffer with mild disease, a proportion of people from all age groups face a prolonged recovery, particularly in relation to the physical activity [41]. Moreover, there is increasing recognition of potential long-term symptoms and complications of 'long COVID', including the enduring malaise and asthenia, cardiopulmonary disease, and psychological sequelae in some people. In this respect, though the current state of understanding of recovery from COVID-19 is limited, the ongoing research has highlighted several key concerns such as the potential for cardiac injury, including post-COVID myocarditis and cardiomyopathy.

Without evidence from robust studies, the current guidelines are based on clinical experience and consensus. A pragmatic approach is recommended for a gradual return to physical activity to mitigate associated risks. In general, all the COVID-19 patients should be risk stratified following recovery before recommending a return to physical activity, which should be gradual, individualized, and based on subjective tolerance of the activity. In general, a return to physical activity should be after at least seven days period free of symptoms, followed by two weeks of minimal exertion. As a rule, those with ongoing symptoms or history of severe COVID-19 need cardio-pulmonary assessment before advising return to physical activity [42].

Post-COVID myocarditis and cardiomyopathy: Most of our data on post-COVID cardiac injury is from patients with severe disease who were hospitalised. This cannot be extrapolated to those who have suffered with mild illness. As such, the incidence of cardiac injury in those with asymptomatic or mild to moderate disease is not known. Cardiac symptoms during illness such as chest pain, palpitations, syncope, severe breathlessness, need assessment for myocarditis and cardiomyopathy, followed by restriction of physical activity and periodic assessment. People who did not require hospital treatment but who had symptoms during their illness suggestive of myocardial injury, such as chest pain, severe breathlessness, palpitations, symptoms or signs of heart failure, or syncope and pre-syncope, should be assessed with a physical examination and considered for further investigations. Both European and US guidelines advocate restrictions on exercise for three to six months in cases of myocarditis confirmed by cardiac magnetic resonance imaging or endomyocardial biopsy.

In a study of in unselected patients who were followed with serum troponin measurements and cardiovascular magnetic resonance imaging after a diagnosis of COVID-19, it was documented that a significant number of patients suffered with ongoing myocardial inflammation several weeks after the diagnosis [30]. The cardiac MR in these patients recently recovered from COVID-19 infection, indicated cardiac involvement in 78% patients and ongoing myocardial inflammation in 60% patients, independent of pre-existing conditions, severity and overall course of the acute illness, and time from the original diagnosis. The study, thus, outlines the need for investigations for cardiovascular involvement and prudence during the convalescent period in advising exercise and return to physical activity.

Post-COVID thromboembolic complications: Pulmonary embolism is associated with COVID-19. Its long-term effects on pulmonary function are not currently known, but the previous relating to the SARS epidemic suggest persistent impairments in pulmonary function and exercise capacity in survivors. An early resumption of physical activity and exercise in the presence of pulmonary dysfunction may be associated with increased morbidity and mortality. Those with respiratory symptoms such as persistent cough and breathlessness are expected to resolve after several weeks, but the progressive, non-resolving or worsening symptoms may indicate pulmonary-vascular complications such as pulmonary embolism, concomitant pneumonia, or post-inflammatory bronchoconstriction and need full cardiopulmonary assessment.

The 'brain fog' and psychiatric symptoms: The 'brain fog' and various psychological sequelae include post-traumatic stress disorder, anxiety, and depression, and psychiatric disorders phenomena, such as psychosis in the post-COVID period. The exercise and physical activity can have beneficial as well as harmful effects. Hence the resumption of exercise and physical activity should be closely monitored.

Activity level and occupational rehabilitation: In the natural course of COVID-19, deterioration signifying severe infection usually occurs following a week from symptoms onset. This being the basis of consensus, a return to regular occupational activity, exercise or sporting activity should only occur after a n symptom-free period of at least seven days. Further, risk-stratification approach is recommended to maximise safety and mitigate risks.

Thus, in practice, for those with mild symptoms during the COVID illness and asymptomatic during convalescence period, there should be a phased return to physical activity with at least a week in between every phase (Figure 5).

The phases have been outlined as - Phase 1: Breathing exercises, mild stretching, and gentle walking. Phase 2: Low intensity walking, mild household and gardening tasks, light yoga. Phase 3: Moderate intensity aerobic and strength challenge. Phase 4: Moderate intensity aerobic and strength challenge with coordination and functioning skills. Phase 5: Return to regular exercise and physical activity pattern. Usually, a light intensity activity is advised for initial two weeks. The Borg Rating of Perceived Exertion (RPE) scale is a subjective assessment of activity and physical work and is helpful in guiding the progress through the phases of increasing physical activity. The patients must rate their subjective feeling of exertion, including shortness of breath and fatigue, on a scale from 6 (no exertion at all) to 20 - maximal exertion [43].

Conclusion: Long COVID's prognostic challenges

In general, levels of physical activity and exercise vary substantially across society. There are various contributing factors in the background and include cultural mores, prevailing concepts of gender, occupational demands, availability of time and cost, access and awareness for physical activity or exercise. As relates to the 'long COVID' syndrome, the inequalities in physical activity have impact on manifestation of various symptoms. In turn, the overall effect of ongoing 'long COVID' symptoms on the patient's life need to be evaluated.

S. N.	Activity phase	Activity details
1.	Phase One	Breathing exercises Mild stretching Gentle walking.
2.	Phase Two	Low intensity walking Mild household Gardening tasks Light yogic exercises
3.	Phase Three	Moderate intensity aerobic Moderate strength challenge
4.	Phase Four	Moderate intensity aerobic Strength challenge with coordination
5.	Phase Five	Return to regular exercise level, Regular physical activity pattern

Figure 5: The phases of physical activity and exercise.

As apparent, the 'long COVID' afflictions are more common than realised earlier. It has been documented in a large survey involving 8200 participants that around one in five COVID-19 patients, continue to have symptoms 5 weeks later, and around one in ten after 12 weeks after infection [44]. According to the National Institute for Health and Care Excellence (NICE) guideline the patients are likely to have ongoing, symptomatic COVID-19 if they present with symptoms 4 to 12 weeks after the start of acute symptoms and have 'long COVID' if they still present with symptoms after 12 weeks [45].

Many of those who have suffered with severe COVID-19 may continue to have distressing symptoms, including breathlessness, anxiety, and chronic fatigue. Symptoms can escalate among patients with co-morbid conditions. Symptomatic or palliative therapy may be an important aspect of overall care [46]. The relief of incapacitating symptoms through provision of holistic care, is another essential component of healthcare for 'long COVID' patients. Further, there is a scope for anticipatory approach to symptomatic management of these patients [47]. The distressing 'long COVID' symptoms are further accentuated by isolation and separation from loved ones, and the anxiety concerning the illness and fear of grave prognosis. The persistent symptoms among COVID-19 survivors pose new challenges to the healthcare providers and may be suitably managed with a combination of pharmacological and non-pharmacological treatments [48].

With the realization of 'long COVID' as a disease entity, the post-COVID-19 care centers are opening at various academic medical centers in the UK, the United States, and elsewhere. They aim to bring together the multidisciplinary teams to provide a comprehensive treatment and coordinated care. The US National Institutes of Health have provided interim guidelines for the medical management of persistent symptoms or illness after recovery from acute COVID-19 [49]. The guidelines and perspective about the overall management of 'long COVID' patients will witness ongoing revisions as new information emerges from research and clinical studies.

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Chapter 7: Ongoing COVID-19 Pandemic: The Lurking Dangers and Pillars of Hope

Background

Introduction – the unrestrained pandemic: The emergence of the novel coronavirus, SARS-CoV-2 in December 2019, has had led to COVID-19 pandemic with devastating consequences. COVID-19, as the disease has unique clinical manifestations which are distinctive, yet bizarre. As the pandemic is spreading, the virus is continuing to evolve. In fact, this process of evolution is a continuum, allowing the virus to adapt to its environment by selecting mutations that make it replicate and transmit more efficiently.

Lurking dangers – the evolving variants: So far, Sars-CoV-2 has infected over million people worldwide and taken on many thousands of mutations. Most of those changes are slow and inconsequential evolutionary dead ends, but can potentially become more transmissible, more virulent, or more resistant to immune response to become unresponsive to the vaccines. Keeping ahead of the pace at which variants are evolving and influencing disease transmission and severity will be key issue for the coming phase of the pandemic.

The pillars of hope – the COVID-19 vaccines: The vaccination remains the most effective tool for protecting from COVID-19. New insights into the functioning of the immune system have made possible the rapid development of new vaccines to combat the raging pandemic and the pathogen and its variants. The vaccines provide us with much-needed hope for the COVID-19 prophylaxis as well as tools to limit the disease severity. World over, the major challenge is to provide equitable access to effective vaccines for masses.

Conclusion – the future COVID-19 scenario: The researchers as well as medical community agree that the vaccination should be continued with the available vaccines. In general, the vaccines induce a more powerful immune response than a natural infection and the mass immunization is crucial to curb the spread of infection, break the transmission-infection cycle and retard the evolution of new variants as well. The ongoing COVID-19 pandemic is a reminder of the need to prioritise health over other aspects of human life, as well.

Uncontrolled-raging COVID-19 pandemic

The infection and SARS-CoV-2 evolution: The emergence of the novel coronavirus, SARS-CoV-2 in December 2019, has had led to COVID-19 pandemic with devastating consequences. COVID-19, as the disease has unique clinical manifestations which are distinctive, yet bizarre. Most persons infected with the virus are often asymptomatic, yet they carry and emit high viral loads, being the major source and transmitters of viral spread. These factors have led to the current perpetuation of the infection, affliction and morbidity, hospitalization, and mortality. The COVID-19 related deaths are now a major cause of death in various countries such as Brazil and the United States, and the United Kingdom.

The world-wide, spread of SARS-CoV-2 has greatly been influenced by masking, social distancing, and lockdown measures. There have been fierce debates about the effectiveness of lockdown measures in containing COVID-19, and their appropriateness in light of the economic and social cost involved. Alfano et al have shown that lockdown is effective in reducing the number of new cases in the countries that implement it, compared with those countries that do not. The lockdown measures are doubtlessly effective in reducing the R0, that is, the number of people infected by each infected person [1]. The discontinuation of major restrictions such as national and international travel and airport reopening, can destabilise the control of the COVID-19 pandemic, though in view of their impact on the economy, various countries are forced to balance the imposing of restrictions against. This situation has been exemplified by the Cyprus experience [2].

However, the differences in morbidity and mortality rates for given prevalence, cannot completely be explained by the practices relating to social distancing, masking, and lockdown measures. The D614G mutant of SARS-CoV-2 after evolving in Wuhan became rampant in various European and South American countries and in the USA. The implications about the clade-G viruses need to be emphasised. Both the older age and SARS-CoV-2 clade-G viral infections could explain 37.43% of the observed variability in cumulative mortality rates across 58 countries [3]. This could be related to the viral genome variations found across the globe and viral haplotype changes in combination with known host factors such as age, race, and presence of comorbid conditions.

In fact, as the SARS-CoV-2 virus infects more people and the pandemic spreads, it continues to evolve. This process of

evolution is a continuum, allowing the virus to adapt to its ecosystem by selecting mutations that potentiate it replicate and transmit more efficiently. Any variation that gives the virus progeny a competitive growth advantage is likely to be selected over the parental genomic virus [4]. With the unrestricted and raging pandemic, the SARS-CoV-2 virus is demonstrating this feature with occurrence of new variants with adaptability and enhanced growth properties (Figure 1).

Whereas a drop in transmission rates means fewer infections, as this is associated with less virus replication leading to fewer opportunities for the virus to mutate. With less opportunity to mutate, the evolution of the virus slows and there is a lower risk of new variants. In due course, this will reduce the chances of re-emergence and future outbreaks of COVID-19, as the population groups develop immunity due to infection or vaccine inoculation.

Thus, to be emphasised, lowering transmission rate is the key to control the pandemic [5]. The control measures such as the use of masks, physical distancing, testing of exposed or symptomatic persons, contact tracing, and isolation have helped limit the transmission wherever they have been rigorously applied. Simultaneously, there have been recurrent outbreaks and re-emergence of the disease in various regions due to laxity of the control measures and unrestricted travels in and outside countries. With the result, various regions have witnessed unmitigated spread of the virus and appear to have lost the grip on control of the COVID-19 pandemic. The persistence and exacerbation of the infection have occurred due to inconsistent adherence to effective public health measures, including wearing masks and maintaining social distancing, the nonavailability of effective treatment, and emergence of the virus variants with increased infectivity and ability to evade immunity.

Factors related to disease transmission: An analytical study in the US has concluded that at least 65% of SARS-CoV-2 infections originate from individuals in the age group 20-49 [6]. Another study of 282 COVID-19 clusters in Catalonia has reported that the viral load was a leading driver of SARS-CoV-2 transmission and people with low viral load infected 12% of their contacts, while people with high viral load infected about twice the number, amounting to 24% of their contacts [7]. In general, the peak in viral load occurs on average a day before appearance of symptoms. Further, it has been documented that the decline in viral load is slower in older patients. Furthermore, the dynamics of viral load following hospital admission is a predictor of morbidity as well as mortality.

There are certain other factors to be considered. A follow-up study has noted that around 5% patients remained persistently PCR-positive after 90 days [8]. However, transmission to close contacts was not observed. It is now clear that SARS-CoV-2 is transmitted predominantly through the air, by people breathing, talking, sneezing, and coughing out virus laden droplets and aerosols. In contrast, transmission of the virus by touching infected surfaces appears to be uncommon.

But impeding the emergence of new variants means doing more of what we know to stop the transmission, such as wearing face masks, social distancing, working from home and tracing infections, and preventive large-scale vaccination. The data from Israel show that vaccine is a powerful tool for curtailing infections and hospitalisations, but alone without the preventive control measures, it may not be a potent defence against the emerging new variants. In fact, a combination of widespread transmission and a partially vaccinated population might push SARS-CoV-2 to acquire vaccine-evading mutations [9].

Distinctive scenario related to COVID-19: Whereas other viruses mutate as they pick up tiny errors in their genetic code when they make copies of themselves, the coronaviruses (CoVs) have evolved to make this copying process more accurate. The CoVs, like the DNA viruses, have the potential proofreading functions as the nsp14 protein acts as a 3′-5′ exoribonuclease on both single-stranded and double-stranded RNA during the viral replication cycle [10]. Through this

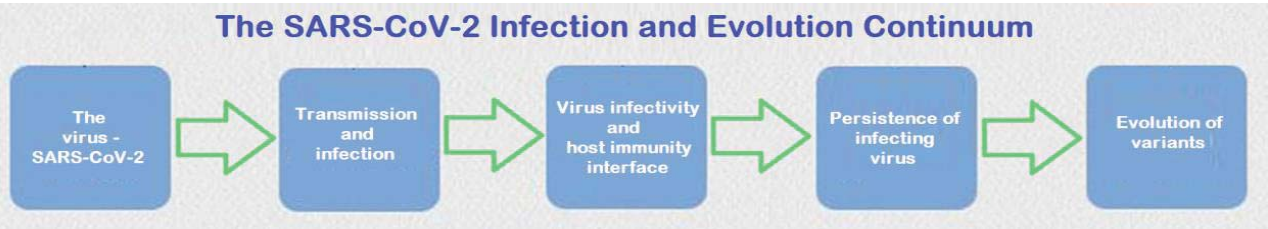

Figure 1: The continuum of SARS-CoV-2 infection and evolution involves the interactions between virus infectivity and host immunity interface - an immunocompromised state leads to persistence of the virus, chronic course of the disease and evolution of mutations and variants.

proof-reading function, the CoVs appear to have overcome the limitation, which in most RNA viruses, during process of replication, lead to the non-viable virions rapidly outnumbering the viable ones, leading to a loss of fitness and/or viral extinction. But the CoVs, like other RNA viruses can resort to RNA viral evolution through recombination involving synthesis of chimeric RNA molecules from two different progeny genomes, and reassortment involving the packaging within a single virion of genomic segments from different progeny viruses [11].

The net result of these mechanisms is that SARS-CoV-2, like other CoVs can spot and correct mistakes in their RNA, which slows down the number of errors that accumulate in their genome. Thus, in evolutionary terms, SARS-CoV-2 virus is a genetic snail. But the same mechanism empowers the virus to develop of more selective and adaptive viable variants. Further, in an uncontrolled situation like an unrestrained pandemic, the multitude of infections presents the virus with endless opportunities to mutate into new variants. That situation is a current reality in the UK, Brazil, South Africa and in dozens of other countries with relatively high levels of transmission leading to emergence of worrisome new variants.

In addition, SARS-CoV-2 is one of several viruses, such as poliovirus, norovirus, and Ebola virus, that can linger for an unusually long period within the human body especially when the host's immune system is compromised. In people with debilitated immune system, the SARS-CoV-2 virus persists in a unique environment [12]. Instead of clearing an infection quickly, an immune-compromised person might only partially wipe out the infection, leaving behind a population of viruses that rebound and replicate to begin the cycle all over again. In such situation, the infecting virus can mutate and evolve to more virulent forms at remarkable speed. There have been documented multiple cases of patients with chronic COVID-19 infections lasting for several months. In general, the natural selection pushes the virus to transmit more easily and acquire survival fitness by become resistant to immune response.

Genomic sequencing and diagnostic assay: From the applied research point of view, understanding the nature of the evolving changes in the SARS-CoV-2 genome provides guidance to develop countermeasures to control the infection as well as diagnostic tests. Further, it is important to monitor the virus for newer mutations which can make it more transmissible or virulent, or both. The random sequencing of the SARS-CoV-2 viruses from patient samples across diverse genetic backgrounds and geographical locations is also a method to look for emerging variants. The genomic sequencing data also guide in developing the diagnostic tests for detecting the virus variants, forecasting likely disease trends, and designing suitable and potent vaccines.

As reported recently, the genomic surveillance, in mid-January, has picked up the E484K mutation in some of the cases with UK variant. The COG-UK dataset (total sequences 214,159) was analysed on 26/01/2021. The S protein mutation E484K (found in VOC 202012/02 B1.351 and VOC 202101/02 P1) has been detected in 11 B1.1.7 sequences. The preliminary data suggest more than one acquisition events [13]. The E484K mutation is also present in the South Africa variant and appear to help the virus evade the immune response. In fact, as the immunity to Sars-CoV-2 nurtures following infection and vaccine inoculation, the virus is pushed to find new adaptations to continue infecting and spreading. We are, thus, witnessing the SARS-CoV-2 virus evolution in real-time. It is feared that with persisting high rate of transmission, the virus will be having greater opportunities to mutate to novel variants and more stable genomic configurations.

The lurking dangers – SARS-CoV-2 variants

SARS-CoV-2 may be the most sequenced virus in history. The first viral whole genome (RNA) sequence information was published on 5th of January 2020 [14]. So far more than 360,000 Sars-CoV-2 genomes have been sequenced and uploaded to GISAID, a platform for sharing viral genomes. So far, Sars-CoV-2 has infected over million people worldwide and carried on several thousands of mutations. Most of these mutations are insignificant and inconsequential evolutionary dead ends. Every time the virus infects a person, it has a novel opportunity to mutate. It can potentially become more transmissible, more virulent, or more resistant to immune response to become unresponsive to the vaccines.

The SARS-CoV-2 variants of concern: The SARS-CoV-2 variants are of concern if they are more transmissible, can cause more severe disease, or likely to evade immune responses to vaccines or make antibody products less effective. In fact, the speed at which variants are evolving and being transmitted will be key issue for the coming phase of the pandemic. Presently, there are four SARS-CoV-2 variants are of epidemiological importance (Figure 2).

Out of the four variants, D614G or the clade-G variant has been present in various regions of the world and having been evolved soon after the beginning of the pandemic for about a year. Presently, three key SARS-CoV-2 variants of

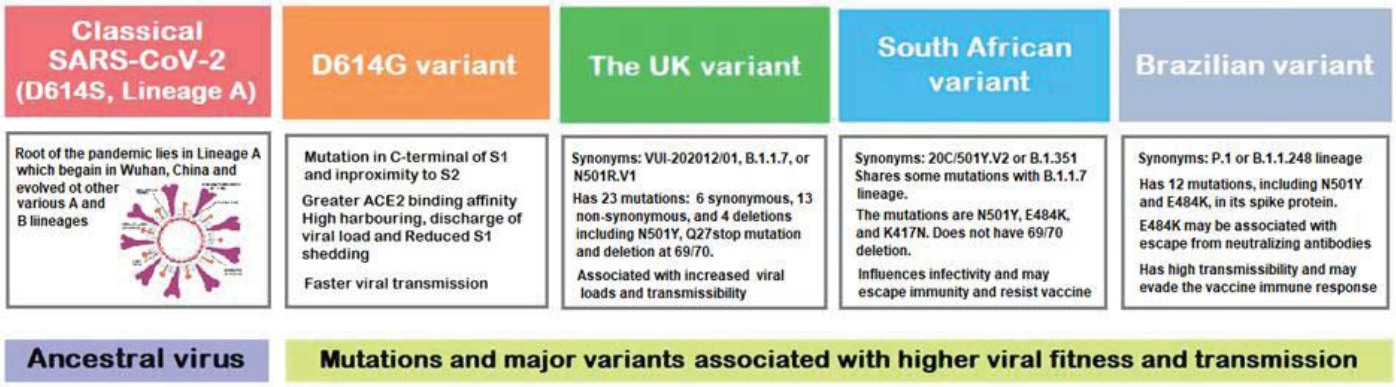

Figure 2: The ancestral SARS-C0V-2 virus and its notable mutations and major variants.

major concern include the variant of B.1.1.7 lineage, first detected in the UK in September 2020; the variant of B.1.351 lineage, was first detected in October 2020 in the Republic of South Africa (RSA); and the variant of P.1 lineage, detected recently in Brazil in December 2020. There has been recent rapid spread of the UK variant or B.1.1.7 and it has now been found in more than 75 countries and spreading locally in Brazil, Canada, China, the United States, and most of Europe.

Being over 70% more transmissible than other variants, the B.1.1.7 is now responsible for the vast majority of new cases in England [15]. The variant bears multiple additional spike protein mutations such as deletion 69–70, deletion145, N501Y (increased hACE2 binding affinity), A570D, P681H (Furin cleavage site), T716I, S982A, and D1118H on the background of the G614 mutation. There have been reported so far 21 cases in the UK of that variant having E484K mutation. The preliminary data suggests that the new variant may be 30% more virulent than others. Further, it is transmitting more readily than other existing variants. Additionally, emerging data suggest that the B.1.1.7 lineage may cause more severe disease [16]. It is being predicted that it is likely to infect most of the world regions in near future [17].

The other variant, RSA variant of B.1.351 lineage, also known as 501.V2, has multiple mutations within two immunodominant domains in the S protein. The variant has been reported in 20 countries including the US, similarly the Brazil variant (P1 lineage) has been found in five countries including the US. The E484K mutation present in South African and in Brazilian variants potentially reduces the effect of neutralising antibodies. There is scientific data to suggest that the new variants may be more transmissible, prompting concerns that they could increasingly affect the populations world over. The distinctiveness of the B.1.1.7 variant which originated in Kent in the UK, points to two likely theories either the virus had mutated abroad and detected once it entered the UK, as most countries do not have a high level of genomic surveillance, or many of the mutations had happened within a single person [9]. As there are travel links with the UK with most countries affected by the new variant, it appears that the country of its origin is the UK. A third remote possibility is that the variant has emerged through recombination of the viral genomes of similar strain and pooling of mutations.

Evasion of immunity by the variants: The new variants evade the human immune response through a process called antigenic variation, whereby the change in the S protein prevents antibodies from binding and neutralizing the virus. The antigenic variation is measured by using known monoclonal antibodies to S protein or using sera from convalescent patients or those vaccinated with an available COVID-19 vaccine. There has emerged data in recent weeks to indicate that the UK variant (B.1.1.7 lineage) as well as the SA variant (B.1.351 lineage) can evade binding and neutralization by monoclonal antibodies to the S protein. Concerning the SA variant (B.1.351 lineage), a study has shown that this lineage exhibits complete escape from three classes of therapeutically relevant monoclonal antibodies and substantial escape from neutralizing antibodies in COVID-19 convalescent plasma. The variant has prospect of reinfection with antigenically distinct variants and may have reduced efficacy to currently available S protein-based vaccines [18].

The fast-spreading SARS-CoV-2 variant B.1.1.7 increases morbidity as well as the mortality risk from COVID-19 compared to previous variants for all age groups, genders, and ethnicities. But as expected, the B.1.1.7 variant does not hit all ages equally but has predisposition to those in the older age-groups. As per the latest analysis the average case fatality rate is around 36% higher for those infected with the new variant. Thus, those aged 70–84, the number who are

likely to die from COVID-19 increases from roughly 5% for those infected with the older variant, to more than 6% for those confirmed to be infected with B.1.1.7. For those aged 85 or over, the risk of dying increases from about 17% to nearly 22% for those confirmed to be infected with the new variant [19].

Presently, the B.1.1.7 has become the dominant variant in the United Kingdom and has spread to other countries. Further, that B.1.1.7 has contributed to an increase in number of patients attending the hospitals and affected the quality of care [20]. Further, there is taking place a convergent evolution and it a small number of patients infected with B1.1.7 variants in the UK have been found to have developed the E484K mutation, which is also found in variants in Brazil and South Africa and may help SARS-CoV-2 partly to evade immunity.

A recent study involving the blood donors in Manaus, Brazil, has indicated that around 76% of the population had been infected with SARS-CoV-2 by October 2020, a proportion of the population well above the theoretical herd immunity threshold. However, the abrupt increase in COVID-19 hospitalisations during January 2021 indicated the higher transmissibility of the P1 variant and/or its potential capacity to escape from immunity [21].

Risk of emergence of SARS-CoV-2 mutations: There is a potential risk of emergence of new mutations during chronic SARS-CoV-2 infection or in lingering course of COVID-19 where treatment over an extended period and immune-compromised state can provide the virus multiple opportunities to evolve. Of particular concern are mutations that have impact on the S protein. The evolution of E484K mutation in the SA and Brazilian variants and potentiating the UK variant of B1.1.7 lineage may hamper effect of neutralizing antibodies. Further, most of the current vaccines in use or under development target the S protein and thus the mutations may affect the efficacy of these vaccines.

Relating to this issue, it has been claimed for the Moderna vaccine that it retains neutralising activity against the UK (B1.1.7) and SA (B1.351) variants. Further, though there was observed a six-fold reduction in virus neutralisation with the B1.351 variant, the titres of neutralising antibodies following two doses of the vaccine were at sufficient levels for protection [22]. As inferred from the preliminary data the mutations in B1.1.7 lineage do not affect recognition by antibodies produced following natural infection or immunisation with the Pfizer vaccine.

A particular variant with the ΔH69/ΔV70 amino acid deletion in part of the S protein appear to make the virus more infectious. The recent research indicates that the ΔH69/ΔV70 deletion by itself can make the virus twice as infectious as the previously dominant variant. Whereas, as shown experimentally, the combined mutations the ΔH69/ΔV70 and D796H made the virus less sensitive to neutralization by convalescent plasma. There is possibility that the D796H mutation alone is responsible for the reduction in susceptibility to the antibodies in the plasma and the role of the ΔH69/ΔV70 deletion is to compensate for the loss of infectiousness due to the D796H mutation [23].

The pillars of hope - the vaccines

Vaccination for disease prophylaxis: The vaccination remains the most effective tool for preventing infectious especially the viral diseases and safeguarding public health. New insights into the functioning of the immune system on a cellular and molecular level have made possible the rapid development of new vaccines. The future holds great promise for vaccine-mediated control of global pathogens and remarkable progress has been made in developing vaccines to combat rapidly emerging and changing pathogens and deal with the pandemics. Although the COVID-19 pandemic is currently raging, the prospects for control of this and future pandemics are bright. The vaccines provide us with much-needed hope, though a major challenge is to provide affordable access to effective vaccines for masses.

Vaccination as a means for COVID-19 prevention is relevant to people of all ages. Further, vaccination can improve individual chances of survival, protect communities from new and re-emerging health threats, and enhance societal productivity. But achieving the promising benefit of vaccination requires much more than the vaccines themselves. There are required timely discovery and development of innovative, effective, safe, and affordable products; effective financing and delivery programs; and credible evidence-based policy recommendations to reassure the public about the value of the vaccines.

It is held that the duration of effective immunity against SARS-CoV-2 will determine at large the pandemic and post-pandemic transmission of the virus. The individual immunity or herd immunity can be achieved either through vaccination or naturally, following recovery from the disease. Further, the effective herd immunity relies on the percentage of the immunized population, the length and effectiveness of the immune response and the stability of the viral epitopes. As the

long-term natural immunity to SARS-CoV-2 is uncertain, individual as well as herd immunity has to be relied on COVID-19 vaccination program and the development of an effective COVID-19 vaccine becomes of paramount importance [24].

The safe and effective vaccines should be given first to those at high risk and later to those at low risk to cover the population to provide immunity at individual and mass level to control the viral transmission. The vaccines are needed to halt the COVID-19 pandemic and to protect persons who are at risk for the infection. Following the successful sequencing of SARS-CoV-2 virus, during January 2020, there has taken place prompt research to develop of COVID-19 vaccines through various vaccine development platforms such as mRNA, protein, viral vector, and others [25].

The vaccine development platforms: There are multiple vaccine approaches utilized by various COVID-19 vaccines under development or given emergency use authorization (EUA). Most of the vaccines developed use the existing vaccine technology. In addition, there are novel genetic vaccine approaches paving the way for more efficient and rapid manufacture of the vaccines (Figure 3). Most of them require two doses to provide protection, and all of them aim to elicit immune response to the S protein of SARS-CoV-2 virus [26].

The traditional vaccine approach -

Inactivated virus vaccines are created by killing or deactivating the virus so that it is unable to replicate in host cells. The whole virus or a subunit of the virus can be used. These vaccines are generally safer than live vaccines as they can be given to everyone, including immunocompromised people. The immune response may not be strong or long-lasting and booster doses, or an adjuvant may be required.

Live-attenuated virus vaccines induce a strong immune response and provide long-lasting immunity. They take longer and are more difficult to mass produce as the virus will have to be grown under enhanced biosafety protocols. Currently only one live attenuated COVID-19 vaccine being developed by Codagenix is registered for Phase 1 human trials.

COVID-19 vaccines using S protein -

In viral vector vaccines the viral vector carries the full-length coding sequence of SARS-CoV-2 spike protein as in Oxford University/Astra Zeneca COVID-19 vaccine (AZD1222), using a modified chimpanzee replication-deficient adenovirus, namely Titi monkey adenovirus ECC-201, and tissue plasminogen activator (tPA) leader sequence. Other viral vectors slowly replicate, carrying SARS-CoV-2 proteins on their surface (replicating viral vectors). Replicating viral vectors best mimic natural infection and hence produce a strong immune response and can be used in lower doses. Human adenoviruses, which cause common cold, have been used as viral vectors. The only one replicating viral vector vaccine candidate in Phase 1 clinical trials, being developed by the Institute Pasteur in France, is now abandoned. The Johnson & Johnson vaccine uses double-stranded DNA and modified adenovirus as carrier. While adenovirus vectors are well tolerated, pre-existing immunity to the viral vector may hamper the immune response to the vaccine.

As per the analysis of the trials for the Oxford /AZ vaccine, the vaccine efficacy after one dose of vaccine from day 22 to day 90 post vaccination was 76%; and, in the group that received two full doses, efficacy was higher with a longer

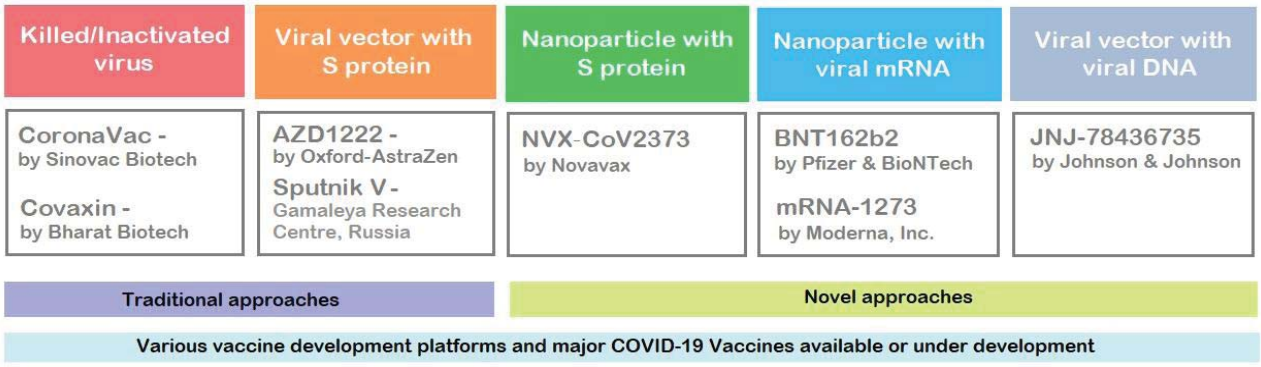

Figure 3: The major vaccine development platforms and approaches.

interval between doses, 82% for a 12-week interval versus 55% for an interval of 6 weeks or less. Further, in the vaccine group after the initial 21-day exclusion period, there were no hospitalisations as compared to 15 in the control group. Compared to the Offord/AZ vaccine, the Russian vaccine, Sputnik V, has an efficacy greater than 90% against COVID-19. Sputnik V works by combining the SARS-CoV-2 S protein into a carrier virus, which is expressed when the virus enters human cells. It uses two different carrier viruses for the first and second doses. shot and the booster shot. Thus, the possibility that subsequent use of same carrier virus could dampen the response to S protein is taken care of [27].

The nanoparticle-based vaccines use nanoparticles in place of viral vector, which are engineered highly stable nanoparticle (nanocarrier). The nanoparticle-S Protein-based vaccines include COVID-19 vaccines being developed by US-based Novavax and Canada-based Medicago which are in advanced Phase 3 clinical trials. Another vaccine, being developed by Flinders University and Vaxine, an Australia biotech Company. The Novavax COVID-19 vaccine is a nanoparticle vaccine containing S protein. As announced by Novavax, its vaccine showed an 89.3% efficacy in its trial in the UK, with over 15,000 participants between 18 and 84 years of age. Of 62 symptomatic cases, 56 (1 of them severe) were in the placebo and 6 in the vaccine group. Over 50% of cases were infected with the B1.1.7 variant, indicating that the vaccine also works against this variant. However, the efficacy dropped to 60% in a smaller trial (4,400 participants) in South Africa, where the B1.3.5 variant is circulating. The efficacy was further low (49.3%) when immune-deficient HIV-positive people were included in the trial [28].

COVID-19 vaccines using genetic approach

The novel genetic vaccine approaches depend on the genetic sequence and more efficient way to produce the vaccine. They may be nanoparticle-based, using an engineered highly stable nanoparticle (nanocarrier). The Pfizer/BioNTech Moderna vaccines using mRNA-nanoparticle technology have 95% and 94.1% efficacy, respectively. Due to the instability of mRNA, these vaccines are difficult to transport and store. The Pfizer COVID-19 vaccine will need storage at -70 °C, shipment on dry ice and will only last 24hrs when refrigerated. Whereas the Moderna COVID-19 vaccine is more stable and can be transported at -20 °C and stored in a standard vaccine fridge (2-8 °C) for 5-days. The COVID-19 mRNA vaccines are only capable of producing S protein and cannot be reversed back into DNA and hence unable to modify it. The mRNA vaccine platform has advantages as a pandemic-response strategy, given its flexibility and efficiency in immunogen design and manufacturing.

The Johnson & Johnson vaccine - JNJ-78436735 or Ad26.COV2.S, uses double-stranded DNA through modified adenovirus, Adenovirus-26 with added gene for the S protein. After the vaccine injection, the adenovirus is engulfed by the host cell. Once inside, the adenovirus travels to the nucleus and leads the CoV gene for S protein to be read and copied into mRNA. The mRNA then leaves the nucleus and, in the cytoplasm, begin assembling spike proteins. Some of the spike proteins thus produced by the cell form spikes that migrate to its surface and stick out their tips. Further, the vaccinated cells may also break up to present on S protein on their surface. The S and S protein fragments are recognized by the immune system. In addition, the adenovirus also provokes the immune system by switching on the cell's alarm systems and activates immune system to react strongly to the spike proteins [29].

The DNA is less fragile than mRNA and the adenovirus's tough protein coat helps to protect the genetic material inside, thus the vaccine can be refrigerated for up to three months at 36–46°F (2–8 °C). The single dose is effective, as the information persists in memory B cells and memory T cells for years or even decades. It has documented 72% efficacy in the United States, 66% in Latin America and 57% in South Africa, 28 days post-vaccination at preventing moderate to severe COVID-19 regardless of age, ethnicity, and presence of comorbidities. It has been claimed to be effective against the SARS-CoV-2 Variant from the B.1.351 Lineage (501Y.V2 variant) observed in South Africa. Further, it is 85% effective in preventing severe disease across all regions studied in all adults 18 years and older 28 days after vaccination. The other DNA vaccines under development include Inovio's COVID-19 DNA vaccine. There are theoretical concerns about potential integration into the vaccine recipient's DNA, but the risk is extremely low [30].

Concerns related to vaccines and eua: The major issue is variable and labile Immunity following the COVID-19 vaccine inoculation, which may not mitigate the SARS-CoV-2 pandemic. In other words, the extent, and the duration of the protective immune response to SARS-CoV-2 may not be adequate. The studies in vaccinated monkeys suggests that SARS-CoV-2 neutralizing antibodies are the primary mode of protection, and the CD8 T-cell response augment the protection, but the duration for which neutralizing antibodies persist is variable. The follow-up studies in the phase 1 mRNA-1273 trial show persistence of neutralizing antibodies for 3 months after the second dose of vaccine. Another issue, it is not

known whether the vaccines can protect against asymptomatic SARS-CoV-2 infection, which is critical to control the pandemic. Finally, the SARS-CoV-2 mutants and variants may be able to escape from protective immune response.

In the view of current understanding of epidemiology and pathophysiology of COVID-19, the development of effective herd immunity seems difficult, as various factors that comprise potential herd immunity may be difficult to be achieved in various population groups in different geographical regions. Further, the exact duration, extent, and effectiveness of herd immunity against SARS-CoV-2 is not established [31]. Nevertheless, it seems that the long-term immunity to SARS-CoV-2 may not be possible in light of our experience about SARS-CoV and MERS. The herd immunity, if possible, may depend only on effective vaccination. Therefore, the development of an effective vaccine is of paramount importance, not only for the control of the current outbreak, but also for the prevention of future outbreaks. Furthermore, the evolving mutations and SARS-CoV-2 variants may dilute the effectiveness of the herd immunity leading to re-emergence of the disease and new outbreaks [32].

The concerns also include adverse events associated with vaccination, likely quality lapses in the manufacturing process, and false alarms regarding vaccine safety [33]. With the availability of new technology, the vaccine-development process is also being condensed and new vaccines are being designed at a fast pace. Maintaining vaccine safety and trust in vaccination process is an increasingly complex public health issue as COVID-19 vaccines are being approved and becoming available in various countries. The unrestrained epidemic involving newer regions requires rapid response including the accessibility of the vaccines before comprehensive safety studies are complete and there arise the need of prophylactic emergency use authorization (EUA).

In response to the pandemic, the efforts for designing, developing and EUA for COVID-19 vaccines have been rapid. By the time the WHO declared COVID-19 a pandemic, biotechnology companies and academic institutions were working on vaccine candidates which included inactivated, live attenuated, S-protein–based, messenger RNA, DNA, viral and nanoparticle vector-based vaccines. In less than a year, the first COVID-19 vaccine-efficacy trials have been completed, and the first vaccines are authorized for prophylactic emergency use. The first vaccine given such authorization has been an mRNA vaccine with lipid nanoparticles (LNPs), BNT162b2 from Pfizer-BioNTech that encodes the prefusion stabilized full-length S protein of the SARS-CoV-2 virus with the overall efficacy 94.1%, for participants 18 to under 65 95.6%, and for those 65 years or older 86.4% [34]. The vaccine begins to protect recipients approximately 10 days after the first dose, with maximum protection after the second dose. The mRNA-1273 by Modena, Inc. was the second vaccine to get EUA. The EUA to other COVID-19 vaccines has followed in various countries.

Vaccinating for COVID-19 prophylaxis

Dosing for COVID-19 vaccines in practice: Most COVID-19 vaccines are given as two injections: an initial 'prime' dose followed by a 'boost' to stimulate the immune system's memory cells and amplify the immune response. There are certain exceptions, such as the JNJ-78436735 vaccine by Johnson & Johnson for which a single dose may be effective. Further, repeat booster doses may be required for COVID-19 vaccines as the immune response seen is a 'labile immunity' and at best will last for 6 to 8 months. Furthermore, in view of emerging more virulent variants, additional vaccine doses are being envisaged.

It has been conjectured and indicated by two small studies that people who have already had confirmed COVID-19 infection earlier, might need only a single dose of a mRNA vaccine [35]. Further, it has been noted that seropositive individuals witness a rapid antibody response after one dose of either the Pfizer-BioNTech or Moderna vaccines [36]. In another small study in HCW with prior COVID-19 infection, it was shown that there occurs a good antibody response to vaccination with IgG spike binding titers rapidly rising by 7 days and peaking by days 10 and 14 post-vaccination [37]. Compared to this, the HCW without h/o previous infection showed significantly lower antibody levels following the vaccination.

Concept of mix and match for vaccines: The vaccine developers during the vaccine design and development phase often combine two vaccines to combat the same pathogen which is termed the heterologous prime boost. The researchers have tried to deploy the same approach against the SARS-CoV-2. In past, a heterologous prime-boost combination was earlier approved by European regulators to protect against Ebola, and experimental HIV vaccines. The concept is being planned to be explored for the vaccines against COVID-19, which are generally recommended to be given as a repeat injection of the same vaccine. It is being conjectured that combining two vaccines could achieve better results than the

individual vaccine and strengthen immune responses by harnessing different mechanisms involved. As per the guidelines the vaccines are not inter-changeable in Britain and the U.S. but can be mixed if the same kind of vaccine is not available for the second dose. Further, the method to mix and match vaccines may make vaccination programmes more flexible and speed up immunization campaigns and reduce the impact of supply-chain disruptions.

In support of the concept, the animal studies have been cited which suggest that a strengthened immune response is possible. Spencer, et al. have reported that the antibody response in mice, following vaccination with a self-amplifying RNA (saRNA) vaccine and an adenoviral vectored vaccine (ChAdOx1 nCoV-19/AZD1222) against SARS-CoV-2 was higher in two dose heterologous vaccination regimens group than single dose regimens group, with the former group showing higher induced titre neutralizing antibodies. Further, the cellular immune response after a heterologous regimen was found to be dominated by cytotoxic (CD8) T cells and CD4 T cells, being superior to the response induced in homologous vaccination regimens [38].

The researchers at Oxford have recently launched a study to test mixing and matching of COVID-19 vaccines by injecting AstraZeneca vaccine followed by the Pfizer vaccine, or vice versa [39]. The Oxford clinical trial aims to enrol 820 people and will test two dosing schedules: one with 4 weeks between the two injections, and another with a 12-week interval. The participants' immune responses will be analyzed after receiving one shot of Oxford-AstraZeneca COVID-19 vaccine and another shot of the mRNA vaccine by Pfizer. The trial by investigators at the University of Oxford, was set to begin enrolment on 4 February. Other vaccine combinations may yield similar results and in fact, Oxford investigators have declared that they will enlist to test combination of the Oxford COVID-19 vaccine with Sputnik V, the Russian COVID-19 vaccine.

The Sputnik V is itself, in a way, a heterologous prime-boost vaccine, consisting of different carrier virus components in the first and second doses. It works by combining two vaccines that tuck the SARS-CoV-2 S protein into a harmless virus, which is expressed when the virus enters human cells and mounts immune response to the S protein. But if the same virus is used in subsequent shots, an immune response against the harmless virus itself could dampen the response to the S protein. Sputnik V addresses this problem by using two different shuttling viruses, one in each shot. Oxford-AstraZeneca's vaccine uses only one, making the heterologous prime-boost studies with Pfizer's vaccine or Sputnik V appealing. The results from the trial arm testing the four-week regimen may be available by June 2021.

Effects of vaccination in real-life scenario: The data from Israel, the country which has vaccinated a large proportion of its population, show that the COVID-19 vaccines are helping to curb infections and hospitalizations among older people, almost 6 weeks after the vaccination drive in that group. Close to 90% of people aged 60 and older in the country have received their first dose of Pfizer's 2-dose vaccine so far. The data collected indicate a 41% drop in confirmed COVID-19 infections in the age group, and a 31% drop-in hospitalization rates from mid-January to early February. In comparison, for people aged 59 and younger, about 30% have been vaccinated in this age group, the cases dropped by only 12% and hospitalizations by 5% over the same time [40]. Further, the difference in case numbers between people older than 60 and younger people was most pronounced in cities where at least 85% of older people had received their first vaccine dose by early January. But the drop in case numbers and hospitalizations might not be solely down to vaccines, as the government had imposed a nationwide lockdown in response to the country's raging epidemic during January 2021. But so far, there is no evidence that vaccinated people, about 40% of Israel's total population. are indirectly protecting unvaccinated people and reducing the onward transmission.

Similarly, a group of researchers in the United Kingdom have noticed early signs that Pfizer's vaccine has contributed to a drop in health-care workers testing positive for the virus. The vaccinated health-care workers were 53% less likely to test positive for SARS-CoV-2 12 days after their first dose than the unvaccinated workers, as per the preliminary results presented in an online webinar on 3 February. The analysis was based on about 13,000 vaccinated people and about 33,000 unvaccinated people who reported their results using a mobile-phone app. This is the first sign in real life, outside trials, about the effect of a single dose. In another study in Israeli health workers, the effectiveness of 51% of BNT162b2 vaccine against SARS-CoV-2 infection 13-24 days after immunization with the first dose was documented [41].

SARS-CoV-2 variants and efficacy of vaccines: As new variants of the SARS-CoV-2 continue to emerge, concerns have been raised about efficacy of the currently available COVID-19 vaccines. Recently, South Africa has temporarily halted the rollout of the Oxford-AstraZeneca vaccine following results from a study showing that the vaccine provided diminished protection against the variant. In this light, we need to religiously follow the public health measures to reduce

transmission and circulation of the virus and the vaccines manufacturers will have to adjust their products to the evolving mutants and variants.

The recent South African study involving 2,026 participants, has indicated that the vaccine was minimally effective at preventing mild to moderate illness caused by the 501Y.V2 variant [42]. As opposed to the overall efficacy of the Oxford-AstraZeneca vaccine being 66% in the larger study that included the UK, Brazil and South Africa, the data from the South African study showed only 22% efficacy against the SA variant.

Whereas a further analysis of the AstraZeneca/Oxford vaccine trial indicates that, although vaccine-induced antibodies were less effective in neutralizing the B1.1.7 variant in experimental studies, its efficacy against symptomatic infections caused by the variant was only slightly lower (75% *vs*. 84%). Still, it is hoped that the vaccine may protect against severe illness, hospitalization, and death. The WHO has a tracking and evaluating tool for COVID-19 variants, which is being expanded to provide guidance to vaccines manufacturers about the changes that may be needed.

There is focus on the T-cell response also. It appears that the T cells could be key to boosting immune response. Experimentally, the RNA vaccines generate powerful antibody responses to the SARS-CoV-2 but fail to stimulate the CD8+ T cells like the Oxford-AstraZeneca vaccine. It has been shown that the CD8+ T cells can strengthen an immune response by identifying and destroying cells infected with the virus. Further, there is a broad functional diversity of T cells, which recognize a broad range of SARS-CoV-2 S protein epitopes, at least 30 to 4o epitopes. These epitopes are separate from the epitopes recognized by the antibodies. This observation raises the possibility that new variants may not be able to escape T cell immunity.

Conclusion - future scenario for the pandemic

The currently available vaccines induce the immune system to produce antibodies that recognize and target the S protein, which is essential for the virus to invade the host cells. Similar to the UK variant, there is accumulation of multiple mutations in the S protein in the SA variant. These mutations allow the virus to attach more strongly to the ACE2 receptor and successfully enter and infect human cells and enhance its transmissibility. There is an emerging possibility that with multiple mutations in the S protein, the COVID-19 vaccines may not be able to generate a strong immune response and protect the recipients from the new variants.

Continuing the COVID-19 vaccination

The researchers as well as the medical community agree that the vaccination should be continued with the available COVID-19 vaccines and there should be a constant effort to get as many people vaccinated and thus protected as possible. In general, the vaccines induce a more powerful immune response to SARS-CoV-2 than what results following a natural infection. The efficacy of the vaccines may be lower than the claimed especially against the variants, still the mass immunization of population groups by the COVID-19 vaccines is important to curb the spread of infection. Curtailing the transmission and breaking the transmission-infection cycle seem to be the only plausible solution not only to control the pandemic, but also to stop evolution of new variants as well.

In addition, there is evidence from studies of sera from individuals vaccinated with the mRNA COVID-19 vaccines suggesting that the vaccines continue to induce a high level of neutralization when tested against the UK variant. When tested against the SA variant, the mRNA vaccines induced a lower level of neutralization, still the vaccines are expected to continue to protect against symptomatic and severe COVID-19 disease [43]. It has been claimed that the mRNA vaccines are 95% effective against symptomatic COVID-19 and nearly 100% effective against severe COVID-19 disease. Therefore, even if the variants cause a modest reduction in the antibody levels generated upon vaccination, the vaccines may continue to provide a significant level of protection against COVID-19. Further, the generation of neutralizing antibodies is one aspect of the immune response to protect against the severe disease, the vaccines also induce T-cell responses, which contributes to protection against symptomatic and severe COVID-19 disease along with the neutralizing antibodies.

Keeping ahead of the future variants

World-over the efforts are going on to limit the COVID-19 pandemic. The WHO is keeping a watch on the epidemic, providing guidance and infrastructure support for the genomic sequencing, and facilitating the availability of COVID-19 vaccines to countries with limited resources through COVAX [44]. The COVAX is co-led by Gavi, the Coalition for Epidemic

Preparedness Innovations (CEPI) and WHO, with the aim to accelerate the development and manufacture of COVID-19 vaccines, and to guarantee fair and equitable access for every country in the world.

The researchers as well as the vaccine manufacturers are monitoring how well the COVID-19 vaccines can control these new variants and searching for the ways for the vaccines to work either by rescheduling the vaccine doses or modifying the vaccine design. In nutshell, we need to keep ahead of future variants of SARS-CoV-2. Moderna, inc. for example, has stated that it will adjust the second or booster injection to match the sequence of the South African variant more closely [45]. With the availability of more genomic sequencing data, the vaccine developers will be able to respond in advance to the evolving variants and major mutations in the SARS-CoV-2 virus population.

The ongoing COVID-19 pandemic is a reminder of the need to prioritise health over other facets of human life. With the fast-changing world with its connected societies and economies, the human population in various countries or regions cannot be regarded to exist in isolation. A local outbreak of a disease, an endemic, or a pandemic in present times demands the global attention. As far as the COVID-19 pandemic is concerned, there is required support for the frontline health workers, help to strengthen the supply chains for health-related products, and boosting for the COVID-19 vaccine availability to the countries with limited resources.

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Postscript: La vie, la calamité, et L' Espoir survivant (The Life, the Calamity, and the Surviving Hope)

Background

Introduction: Ongoing COVID-19 calamity: As a disease, COVID-19 is still in the pandemic phase because infections continue to increasingly occur world-wide and various population groups are still susceptible. It is likely that the SARS-CoV-2 will not be eradicated but become endemic and continue to circulate and cause infections in pockets of the global populations for years to come. It may evolve into more transmissible and virulent forms with novel mutations and variants, and associated factors may worsen the overall scenario with involvement of newer population groups and world regions.

Mutations, variants, and immune escape: The unabated prevalence increases risk of mutations, as the virus has more chances to mutate. Further, in areas where the incidence rates are high, selection pressures favour the emergence of variants that evade neutralizing antibodies. Furthermore, as population groups receive vaccination, immune pressure is conjectured to facilitate and speed up the emergence of such variants by selecting for escape mutants. In due course, these selected variants would replace previous versions of the virus propelling the pandemic or the endemic disease later on.

Associated uncertainties with SARS-CoV-2: There are various associated uncertainties with the SARS-CoV-2 virus and the disease it causes. Due to evolving genomic changes, the virus elicits erratic and labile immune response. Simultaneously, the host factors are highly variable and largely uncontrollable. Further, the control measures and available vaccines for COVID-19 may not reduce the prevalence of infections drastically for multiple reasons. These epidemiological drivers would lead to persistence of the virus and endemicity of the disease interspersed by periodic outbreaks and re-emergence.

The human life during COVID-19 pandemic: With COVID-19 becoming an endemic disease, the SARS-CoV-2 virus would be first encountered during childhood, typically causing mild manifestations or none. The population groups will develop some immunity through natural infection or vaccination and may not suffer with severe illness, except in those with comorbid conditions or immune-compromised states, and the disease course would depend on evolving variants, efficacy of vaccines, and nature of immunity to the virus. The herd immunity against SARS-CoV-2 may remain a myth and with individual immunity being labile and waning after 6-8 months, booster doses of updated vaccine will be required at regular intervals.

Future scenario and search for solutions: To mitigate the spread of SARS-CoV-2 virus, various countries have implemented a wide range of control measures from time to time and likely to resort to, in future as well. There is need for genotyping and genomic sequencing capability for quick and effective utilization of epidemiological data. Simultaneously, the large deployment of COVID-19 vaccines under way needs a rapid and effective global effort. The next-generation vaccines may stimulate T cells effectively, apart from generating antibodies against the virus, and there is possibility of designing a universal coronavirus vaccine or pan-virus vaccine for immunization against multiple variants and strains. On the therapeutic side, use of probiotics as adjuvant therapy may Improve the prognosis and clinical outcomes in COVID-19.

The resilent virus and ongoing pandemic

Over hundreds of coronaviruses infect bats, pigs, camels, dogs and cats, and other animals. The seven types of coronaviruses infect humans, the first four human coronaviruses include HCoV-229E, HCoV-OC43, HCoV-NL63 and HCoV-HKU1, which cause mild to moderate upper-respiratory tract illnesses. Whereas other three aggressive human coronaviruses capable of causing serious morbidity and even mortality, have emerged during the last two decades and include SARS-CoV, MERS and now SARS-CoV-2.

The influenza pandemic, which afflicted humanity in 1918 with fatality over 50 million people world-wide is yardstick by which all other pandemics are compared [1]. The pandemic was caused by influenza A virus, which originated in birds. Since then, all the later influenza epidemics have been caused by descendants of the 1918 virus. Presently the influenza virus descendants circulate the globe and infect a significant number of people each year. The influenza epidemic occurs

when a population group is naive to the virus and with the population groups developing immunity to the virus, it may become a seasonal disease. This is well exemplified by swine flu caused by influenza A virus subtype H1N1 in year 2009-10.

As a disease, COVID-19 is still in the pandemic phase because infections continue to increasingly occur world-wide and various population groups remain susceptible. It is expected the SARS-CoV-2 will not be eradicated but become endemic, continuing to circulate in pockets of the global population for years to come and causing outbreaks in regions where it had been earlier eliminated [2]. The optimistic view holds that the impact of COVID-19 on the humanity in terms of social isolation, morbidity, and mortality may lessen, as the population groups acquire some immunity through natural infection or from vaccination and the disease becomes endemic. Whereas the pessimistic view holds that with ongoing evolution of new mutations and variants and other variables may worsen the scenario with involvement of newer population groups and world regions (Figure 1).

If vaccines are able to block transmission and effective against novel variants it may be possible to achieve herd immunity in regions where enough number of people are vaccinated. It has been shown that a vaccine that is 90% effective would need to reach at least 55% coverage to achieve temporary herd immunity with social distancing measures, face masking, and a large number of people working from home. The similar vaccine would need 67% coverage or even higher levels if the vaccine is less than 90% effective at blocking transmission or if there is increased transmission because of a new variant, to provide herd immunity if the control measures are not followed or lifted [3].

A large study has shown that levels of neutralizing antibodies start to decline after around six to eight months following SARS-CoV-2 infection. If a new infection arises, the memory B cells can produce antibodies and T cells that can eliminate virus infected cells, but it is not established whether this immune memory can block the viral reinfection. It could take years or even decades to reach a state where enough of the population has sufficient immunity. Further, allowing the virus to spread unchecked to that point may result in millions of deaths world-wide, so the immunity through vaccination is the choice.

In fact, there are various uncertainties associated with the virus, SARS-CoV-2 and the disease, COVID-19 it causes. The virus has a large genome and prone to mutations, yet simultaneously, it carries the nsp14 protein, which acts as a 3′-5′ exoribonuclease and has proofreading functions during the viral replication cycle. The immunity developed to the virus appears to be short-term and labile, lasting for 6-8 months at most and with evolving mutations and variants, the virus can potentially evade or escape immunity acquired following natural infection or by vaccination [4].

Mutations, variants, and immune escape

Mutations and evolution of new variants: The higher number of cases increase risk of mutations, as the virus has more chances to mutate. Further, in areas where the prevalence of the virus is high, selection pressures favour the emergence of variants that evade neutralizing antibodies. Further, once a large population receives vaccination, immune pressure is conjectured to facilitate and speed up the emergence of such variants by selecting for escape mutants. Thus, in its gradual course, the disease will evolve into a phase of mutations and emerging variants. Further, the new variants

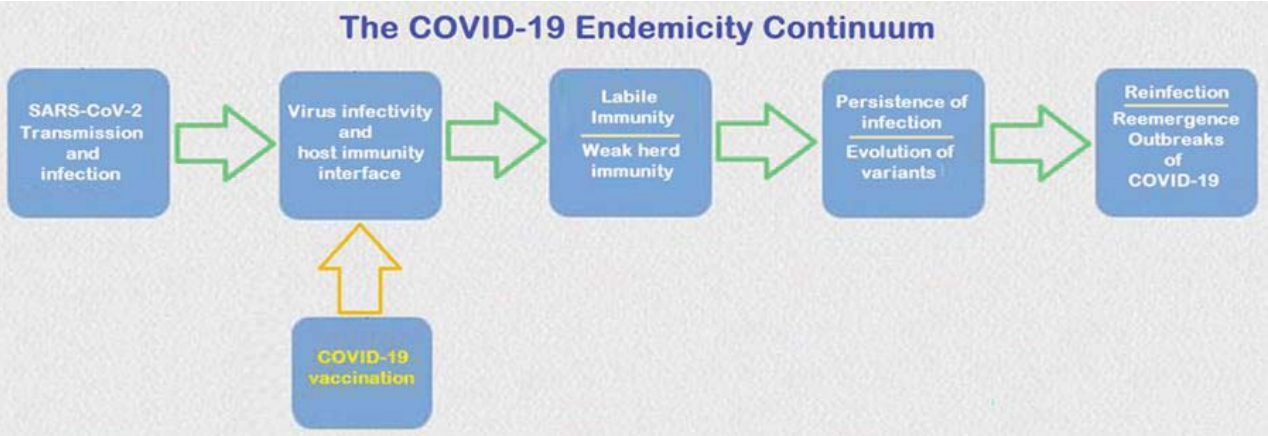

Figure 1: The COVID-19 endemicity continuum.

are likely to have mutations in the parts shown to increase the ability of the virus to infect cells. SARS-CoV-2 may evolve to escape immunity, like the historical evolution of human coronavirus 229E [5]. In due course, these selected variants would replace previous versions of the virus propelling the pandemic or the endemic disease later on. Considering the inherent epidemiological features, COVID-19 may remain a serious endemic threat for years to come or even decades.

The SARS-CoV-2 virus undergoes mutations in its genome, crucially affecting the S protein frequently targeted by antibodies. Compared to the current emerging variants, the earlier D614G mutation increased the transmission ability of the virus compared to the ancestral virus without the mutation. When the mutation slices out a section of the genome, the change is called a deletion. Various studies have shown that deletions tend to occur mostly at few distinct sites in the genomic region coding for S protein. McCarthy, et al have identified more over 1,000 samples with deletions in the genomic region [6].

The important variants of SARS-CoV-2 are -

B.1.1.7 lineage (The UK variant, VOC202012/01, 20I/501Y.V1) carries a mutation in the RBD of the S protein at position 501, replacing asparagine (N) with tyrosine (Y), termed as N501Y, which appears to help the virus spread more easily. The mutation is also seen in SA variant and in Brazilian variant, P_1. In addition, the variant has 69/70 deletion resulting in a conformational change in the S protein and P681H mutation near the S1/S2 furin cleavage site. The variant can potentially evade detection by viral diagnostic tests, has an enhanced transmissibility, and a probable heightened mortality risk. There has been found a new variant B1525 in the UK, which has genomic similarities to the B117 variant and contains a number of mutations including the E484K mutation to the S protein.

B.1.351 lineage (The SA variant, 20H/501Y.V2) carries several mutations in the spike protein, which include K417N, E484K, N501Y, but not the 69/70 deletion. It has E484K mutation, having an impact on neutralization by some polyclonal and monoclonal antibodies, leading to immune escape. The early results suggest that the T cells could be less vulnerable to the mutation as T-cell responses to COVID-19 vaccination or previous infection do not target the mutated regions. Thus, if T cells remain active against the 501Y.V2 variant they might protect against severe disease.

P.1 lineage (Brazil variant, 20J/501Y.V3) has 17 unique mutations and three mutations in the S protein RBD, including K417T, E484K, and N501Y, which may have an impact on transmissibility and antigenic profile affecting the potential of antibodies produced by a natural infection or vaccine and diminished susceptibility to therapeutic agents like monoclonal antibodies. The P2 variant occurs throughout Brazil, but unike the P1 variant, it is not yet clear whether P2 leads to any change in the course of illness. The most widely disseminated SARS-CoV-2 Brazilian lineage B.1.1.33 that evolved from an ancestral clade, here designated B.1.1.33-like. The B.1.1.33-like lineage may have been introduced from Europe or may have arisen in Brazil in early February 2020 and a few weeks later gave origin to the lineage B.1.1.33 [7].

The driving factors for COVID-19 endemicity: There are multiple associated uncertainties with the SARS-CoV-2 virus and the disease it causes. The virus is evolving through mutations and genomic changes, it elicits erratic and labile immune response, and various factors including the host factors are variable and to some extent uncontrollable, make it difficult to predict the course of the disease in the current scenario. Further, the control measures and available COVID-19 vaccines do not appear to reduce the prevalence as well as the severity of infections drastically as the result of appearance of viral variants which can evade immunity and lead to persistence of the virus resulting in periodic outbreaks and reemergent epidemics [8].

In an endemic disease phase, the infections become relatively constant across years, with occasional flare-ups. It is being conjectured that SARS-CoV-2 may follow a similar course and with time, the COVID-19 may become a much less serious challenge and evolve into a seasonal disease interspersed with intermittent outbreaks. Over time covid-19 may become a disease first encountered in early childhood, typically causing mild infection or none at all. But this optimistic turn of events is not being heralded so far.

There are certain epidemiological driving variables leading to persistence of the virus and endemicity of the disease interspersed by periodic outbreaks and re-emergence (Figure 2).

The evolutionary selection pressures on SARS-CoV-2 are not geographically specific. In fact, the virus is encountering similar selection pressures wherever it is transmitting and has relatively high prevalence. Thus, the selection for random mutations that can confer a fitness advantage for the virus can happen anywhere, and at any time. The speed and success

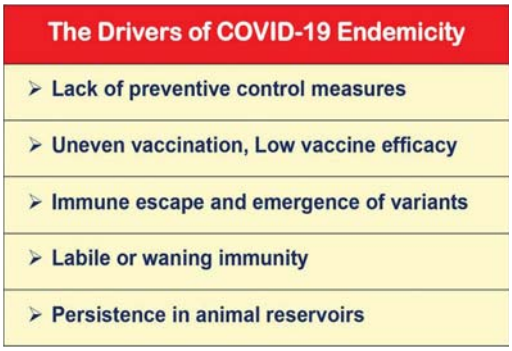

FIgure 2: The drivers leading to the endemicity of COVID-19.

of vaccination may impose another selection pressure on the evolution of the virus, leading to selection of new mutations and emergence of novel variants with ability to persist and propagate.

The immune escape is supposed to be a major driver for the virus's continuing circulation. There is decreased neutralization of variants due to antigenic evolution of the viral S protein, especially in the receptor-binding domain (RBD). Immunity from past vaccination or infection should blunt the disease severity as with various endemic coronaviruses, but the accumulated mutations to SARS-CoV-2 may significantly erode neutralizing antibody immunity and the efficacy of SARS-CoV-2 vaccines. Further, if the vaccines do not stop some people from developing severe disease manifestations, the virus will continue to be a significant disease burden. A similar situation could emerge if a large number of people decline COVID-19 vaccines.

The variants B.1.1.7, B.1.1.298, or B.1.429, continue to be potently neutralized despite the presence of mutations in RBD [9]. Other SARS-CoV-2 variants may escape vaccine-induced humoral immunity. The P.2 variant, which contains an E484K mutation within the RBD region, is capable of significantly reducing the neutralization potency of fully vaccinated individuals. Similarly, the P.1 strain, which has three RBD mutations, can more effectively escape neutralization. The B.1.351 variant exhibits remarkable resistance to neutralization, largely due to three mutations in RBD but with a measurable contribution from non-RBD mutations [10].

As documented, the efficacy of vaccines fades in the face of the 501Y.V2 variant. At least three vaccines, by Novavax, Johnson & Johnson, and Oxford-AstraZeneca vaccines are less effective at protecting against mild COVID-19 in South Africa, where the 501Y.V2 variant dominates. For Oxford- AstraZeneca vaccine, the efficacy was reported to be only 22% effective against mild COVID-19 in a sample of 2,000 people in South Africa. The newly emerging and fast-spreading SARS-CoV-2 variants can also potentially reduce the protective effects of the mRNA COVID-19 vaccines by Pfizer–BioNTech and Moderna, Inc. [11]. As noted, within 3–14 weeks after the second dose, those inoculated developed several types of antibodies, including some that can block SARS-CoV-2 from infecting cells, but the antibodies were only one-third as effective at blocking the mutated variants.

There is another potential possibility of the SARS-CoV-2 virus establishing itself in animal reservoirs. The endemicity of the disease will be influenced by its persistence in animal population, as evidenced by the outbreaks in mink farms [12]. The SARS-CoV-2 virus has been shown to be able to pass to and fro between minks and handlers, similar to several other viruses related to diseases such as yellow fever, Ebola, and chikungunya, where the viruses persist in animal reservoirs and find chances to spill back.

The associated uncertainties with COVID-19

The immune response to infection and vaccines: The concern about the SARS-CoV-2 variants that are partially resistant to antibody defences has spurred a renewed interest in other immune responses that may protect against the viruses. In case of the SARS-CoV-2 variants, where the antibodies generated following vaccination may become less effective, the cellular immunity can help to maintain an enduring immunity. In particular, the T cells response is important and appears to target and destroy the virus-infected cells.

The immune system produces mainly two types of the T cells in response to the infecting viruses. There are killer T cells

(CD8+ T cells), which detect and destroy the virus infected cells, and the helper T cells (or CD4+ T cells), which stimulate the production of antibodies against the virus and killer T cells. As apparent, the T cells do not prevent infection, because they come into action only following the viral infection but play a role in clearing the viral infection. In the projected scenario in case of COVID-19, if CD8+ T cells are able to remove the virus-infected cells before they spread further from the upper respiratory tract, there will be restricted transmission due to reduced harbouring and shedding of the virions. This may reduce the severity of disease manifestations from severe to mild, as well.

The T cells may be more resilient than antibodies to threats posed by evolving mutations and emerging variants. Following SARS-CoV-2 infection, there are generated T cells targeting at least 15–20 different fragments of viral proteins. In addition, the T cells can target viral proteins expressed inside infected cells, some of which are essentially stable. But the protein snippets used as targets can vary widely from person to person and there evolve a large variety of T cells acting against the virus, hindering the mutations and immune escape, unlike the situation for antibodies. The immune system through the T cells is able to recognize SARS-CoV-2 in multiple ways.

Various studies have reported a robust antiviral T cell response in adults recovered from COVID-19 (13). The observed robust T cell response is against the S protein as well as other viral proteins. The circulating SARS-CoV-2–specific $CD8^+$ and $CD4^+$ T cells have been identified in ~70% and 100% of COVID-19 convalescent patients, respectively and correlate with the anti-SARS-CoV-2 IgG and IgA titers. The M, S and N viral proteins account for 11-27% of the total $CD4^+$ response, with additional responses to nsp3, nsp4, ORF3a and ORF8, among others [14]. There has been done a comprehensive analysis to establish the patterns of immunodominance of different SARS-CoV-2 antigens and virus-specific CD4+ and CD8+ T cells, and SARS-CoV-2 epitopes in COVID-19 cases [15]. Further, both natural infection with SARS-CoV-2 and immunization with COVID-19 vaccines induce protective immunity but the vaccine-induced immunity is more robust heterotypic immunity than natural infection to emerging SARS-CoV-2 variants of concern [16].

Viral genomic and phylogenetic analyses: In general, the SARS-CoV-2 variants emerging in a range of geographical locations seem to share certain mutations. The non-synonymous substitutions affecting S protein are common and affect a number of SARS-CoV-2 lineages. The repeated evolution of a trait in independent populations provides strong evidence of adaptation. The coincidental rise and spread of variants on separate genetic backgrounds is remarkable and suggests some fitness advantage. As such the mutations may enable the virus to escape from neutralizing antibodies or enhance transmission through increased affinity for the ACE2 receptors or similar mechanisms.

The genomic surveillance in the US has detected a rapid rise of numerous clade 20G (lineage B.1.2) infections carrying a Q677P substitution in the S protein. The phylogenetic analyses have revealed evolution and spread of six distinct Q677H sub-lineages, from the samples collected between mid-August to late November 2020. There are four 677H clades from clade 20G (B.1.2), 20A (B.1.234), and 20B (B.1.1.220, and B.1.1.222), and a pair of clade 20G clusters in varying number of cases. There is rise of S:677 polymorphic variants, with the mutations in proximity of the polybasic cleavage site at the S1/S2, which is consistent with its functional relevance during cell entry. The evolution of the trait may confer an advantage in viral transmission.

In a recent study, Hodcraft, et al. have reported seven new variants of the SARS-CoV-2 virus in the US. The researchers are tracking virus variants and some of them may more easily transmissible and more virulent than the ancestral virus and can affect the efficacy of vaccines. The researchers have named a new variant, Robin (first spotted in October), accounting for 27.8% of sequenced viruses in Louisiana and 11.3% in New Mexico between the start of December 2020 and mid-January 2021 [17]. The researchers have identified six other variants with a mutation at the same position in the S protein. Interestingly, the variants have been named after American birds, as Robin 1 and Robin 2, Pelican, Yellowhammer, Mockingbird, Bluebird, and Quail.

There is some proof that the vaccines spur antibody surge against a COVID variant, as well. One shot of either the Moderna or the Pfizer vaccine provokes a strong immune response against an emerging variant of SARS-CoV-2, according to tests in people who have recovered from COVID-19 [18]. In the study, before inoculation, nine of the ten individuals had neutralizing antibodies against the original virus, although the levels generated were highly variable. Antibodies from only five people could neutralize B.1.351. Following a single shot of the vaccine, however, participants' levels of neutralizing antibodies against both forms of the virus increased by approximately 1,000-fold.

Mapping of viral mutations with immune escape: The global surveillance of genomic changes in SARS-CoV-2 varies

widely, with leading countries such as Australia, New Zealand, the United Kingdom, and Denmark sequencing viruses from 5-50% of all cases and lagging countries such as the United States, France, Spain, and Brazil sequencing less than 1% of all cases. The mapping of viral mutations and variants with Immune escape capability, identifies mutations that can escape antibody binding. Many of the mutations that escape single antibodies are found to be circulating in the human population in various regions. Further, many escape mutations do not impair the RBD folding or ACE2 affinity and that some are already present at low levels among circulating viruses.

Therapeutically, the anti–SARS-CoV-2 antibodies against viruses can be rendered ineffective by mutations that are selected during treatment of infected patients or that spread globally to confer resistance on entire viral clades. Therefore, determining which SARS-CoV-2 mutations escape key antibodies is essential for assessing mutations observed during viral surveillance [19]. The mapping of variants with Immune escape capability has scope for developing novel therapeutic modalities to deal with the COVID-19 pandemic and can help in developing effective anti–SARS-CoV-2 antibodies to target the RBD region of S protein which mediates binding to the ACE2 receptors.

Evolving scenario and expected course

The projected course for COVID-19 pandemic: The prime scenario, which seems to be the most optimistic eventuality is the virus persisting with COVID-19 becoming an endemic disease. The population groups will develop some immunity to through natural infection or vaccination and may not suffer with severe disease, except in those with comorbid conditions or immune-compromised states. As occurs with other four endemic coronaviruses, OC43, 229E, NL63 and HKU1, responsible for roughly 15% of respiratory infections, the SARS-CoV-2 virus may be first encountered in early childhood when it typically causes mild clinical manifestations or none.

The other likely scenarios would depend on the evolving variants and the pattern of immunity to SARS-CoV-2 in long run. A large study of people who have had COVID-19 suggests that their levels of neutralizing antibodies which help to block reinfection, start to decline after around six to eight months [20]. But apart from the antibodies, those infected or vaccinated develop T cell response as well, which help in eliminating the virus-infected cells. They also develop memory B cells, which can produce antibodies in event of a new infection. But it is not established that the immune memory can block viral reinfection. The cases of reinfection are occasional but may become more likely with the evolving SARS-CoV-2 mutations and variants which can escape immunity. Further, immunity being labile, may wane entirely after 6-8 months or a year or two.

The SARS-CoV-2 infection has spread around the world and may seem like an endemic disease. But, in the endemic phase of the disease, the number of infections should become relatively constant across years, with occasional outbreaks. Because the disease continues to increase in prevalence worldwide, and with various population groups in world regions being still susceptible, technically COVID-19 is in a pandemic phase [12]. It is being conjectured that to reach the steady state of endemicity for COVID-19 it may take a few years or even a decade.

Natural infection, vaccination and herd immunity: Depending on the way human populations develop immunity to the virus, will determine the course of the disease. Allowing the potentially dangerous SARS-CoV-2 virus to spread unimpeded may be the fastest way to develop herd immunity but that would result in millions of deaths world-wide. Further, for the SARS-CoV-2 virus, herd immunity may be a myth as the immunity ensuing natural infection is not a lasting immunity and may not prove to be a defensive immunity. Hence, the goal is to ensure a protective immunity in the human population through COVID-19 vaccination.

The countries that have begun vaccinating their population for COVID-19 are likely to witness a reduction in the severe manifestations, as seen in Israel [21]. But how effectively the vaccines can reduce the transmission of the virus will depend on various factors. There are data from clinical trials to suggest that vaccination may prevent symptomatic disease as well as transmission from a person to other. On an optimistic note, if the vaccines can prevent transmission and remain effective against the new variants as well, it may be possible to eliminate the virus in regions where enough people are vaccinated to protect those who are not, contributing to herd immunity. It is being speculated that a vaccine that is 90% effective at blocking transmission will need to reach at least 55% of the population to achieve temporary herd immunity as long as some social distancing, work from home and masking are followed. For a vaccine less effective than 90%, vaccine coverage should be greater to blunt the SARS-CoV-2 circulation.

Conclusion: protecting from COVID-19

Control measures/non-pharmacological interventions

Various governments across the world have implemented a wide range of control measures and non-pharmacological Interventions (NPI) to mitigate the spread of SARS-CoV-2 virus. It may be important to understand and assess their relative effectiveness. The measures have included closing all educational institutions, limiting gatherings to 10 people or less, and closing face-to-face businesses have been effective in reducing transmission considerably. It has been found that the additional effect of stay-at-home orders was comparatively small. The ultimate goal of the NPI or control measures including partial or more extensive lockdown is to reduce the spread, prevalence, morbidity, and excess mortality of COVID-19.

Closing the nonessential face-to-face businesses was only somewhat more effective than targeted closures, which affected businesses with high infection risk, such as bars, restaurants, and clubs. Closing both schools and universities was found to have a robust effect. Several measures were associated with a clear reduction in the effective Reproduction Rate, Rt. Further, there is evidence that the control measures are effective at mitigating and suppressing outbreaks of COVID-19 [22].

Advances in vaccine technology and pan virus vaccine

With the raging COVID-19 pandemic, there is required rapid genotyping and genomic sequencing capability to be applied in real-time to the positive samples for quick and effective utilization of the data. The large and most rapid deployment of COVID-19 vaccines under way, should be equitable and needs a global effort. Most COVID-19 vaccines, including the Pfizer-BioNTech and Moderna vaccines, are able to decrease symptomatic infection in real world. The studies indicate that the mass vaccination campaigns are helpful in reducing the burden of symptomatic disease. Further, with vaccination, there is appreciable reduction in the rate of serious illness. Still there is a continuing risk that the virus mutations and novel variants may in various ways render the existing COVID-19 vaccines less effective.

Some COVID-19 vaccine developers are looking at ways to develop next-generation vaccines that stimulate T cells more effectively. The S protein-based vaccines carry the risk that the S protein is fairly variable and prone to mutating, leading to their reduced efficacy. Whereas the antibodies detect only the S protein which decorates surface of the virus, the T cells can target viral proteins expressed inside infected cells, some of which are very stable. This raises the possibility of designing vaccines against proteins that mutate less frequently than the S protein and incorporating targets from multiple proteins into one vaccine.

The recent technological advances in biomedical, computing, and engineering sciences has ushered in a new era in antigen and vaccine discovery. Simultaneously, the technology has potential to accelerate identification of common antigenic targets shared across coronaviruses. Databases of genetic sequences of various coronaviruses can be used to model the evolutionary emergence of the viruses. Further, the ongoing efforts to decode the principles of immunity in aging populations can enhance the effectiveness of vaccines for elderly. There is a possibility of developing a universal coronavirus vaccine, which is scientifically feasible [23]. There has been suggested an effort to put resources for developing 'pan-virus vaccines' that can provide immunization against multiple strains of a virus. This is specially required in the context of SARS-CoV-2, which is mutating and evolving to more transmissible and virulent forms.

Probiotics as adjuvant therapy for COVID-19

It is indicated from various studies that the use of probiotics as adjuvant therapy may Improve the prognosis and clinical outcomes in COVID-19. During the in course of infection process, there is modulation of virus infectivity by the commensal microbiota of the host. In the regulation of viral infection, commensal microbiota It may promote viral infectivity by facilitating genetic recombination of viruses and enhance their infectivity and other diverse mechanisms. But microbiota can also exert substantial inhibitory effects on viral infection. In addition to fostering the generation of immunoregulatory Treg cells, the commensal microbiota has antiviral effect by suppressing the activation of effector immune cells and by inhibiting the production of various inflammatory cytokines that are pivotal for virus elimination. In addition, the beneficial probiotic bacteria are demonstrated to promote the host defence and to improve immune response [24].

The SARS-CoV-2 infected patients are often administered antibiotics and antivirals, which could also result in gut microbial dysbiosis. The use of probiotics, and their metabolites SCFAs (short-chain fatty acids), may reinforce innate and adaptive immunity in SARS-CoV-2 patients. Further, the administration of probiotics may increase anti-inflammatory

cytokines, decrease proinflammatory cytokines, improve antiviral antibody production, and reduce the viral load. The probiotics administration may help in reducing SARS-CoV-2 dissemination in the respiratory tract and gut, reinforcing both anti-inflammatory responses and immune defences, and thus can be an effective adjuvant strategy against the clinical complications [25].

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