Background

Many emergent and developed nations are facing an immeasurable strain of infectious diseases, even though vaccines have tamed most of the diseases, newer and stemming infectious diseases, such as SARS, Ebola, Nipah, Hantavirus, Dengue, Chikungunya, Rocky Mountain Spotted Fever, West Nile virus infection, and so on are still a major health threat globally. Since December 2019, a conglomeration of cases of pneumonia of unidentified origin were picked up in the Hubei and Wuhan provinces of China. The World Health Organization declared a Public Health Emergency Of International Concern (PHEIC) over this global pneumonia (Covid - 19) outbreak on 30th January 2020.[1]

The pathogen recognized was a novel beta coronavirus that is referred to as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), which showed resemblance with SARS-CoV, phylogenetically.[2] As of 22nd December 2020, the coronavirus was affecting 220 countries and territories worldwide with a total count of 77,718,154 cases out of which 21,415,746 are currently infected individuals.[3]

The review endeavors to amalgamate the original research work on beta coronaviruses highlighting its phylogenetic relationship with the previous strains, virology, pathogenesis of Covid-19, and how we can direct our study to conclude this pandemic earlier.

Keywords: Coronavirus, Covid-19, pandemic, pathogenesis, SARS-CoV-2

Abstract

The novel disease of 2019 by name coronavirus (Covid-19 or SARS-CoV-2), was first detected in December of 2019 in the Seafood Market of Huanan in Wuhan region of China. In less than a month it was proclaimed as a pandemic by The World Health Organization (WHO). Now, even after a year, it still remains to be a concern as a jeopardy to the global public health. The inception of Covid-19 has been identified as the third encounter of a highly morbid coronavirus after Severe Acute Respiratory Syndrome (SARS-CoV) and Middle East Respiratory Syndrome (MERS-CoV) causing coronaviruses, in merely two decades. In this review, we illustrate the general features of coronavirus and highlight the pathogenesis of the disease, bringing forth the different theories of disease progression, which may help clinicians and other health professionals to achieve a direction for further research, therapeutic protocols and development of the vaccine for combating SARS-CoV-2 infection.

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Evolution

Covid-19 has been the third epidemic in the past two decades caused by coronavirus after severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS). In 2003, a new coronavirus, also originated from south east China, specially from the Guangdong province, which presented as bizarre pneumonia and it was named as SARS coronavirus that met the Koch’s postulates. Through the years, although the medical facilities have made monumental improvements, there has been no definite treatment or even a vaccine for SARS. In 2012, there was an emergence of another outbreak in the Middle East of novel coronavirus with similar features of SARS in 2003. Both SARS and MERS were caused by coronaviruses with mortality rate up to 10-15% and 37%, respectively.

There is corroboration that initial SARS pandemic originated in China, in bats and it propagated to humans, most probably after a jump to an intermediate host, the civet (Paguma larvata). Likewise, MERS_CoV was endemic to Somali or Arabian camels, called the dromedary in the Middle-East. It could have been transmitted to humans from these camels, but its origin from bats is also not denied. The genome sequences of the 2020 pandemic, Covid-19 indicate that it too may have had an origin from Chiroptera, though it is proposed that there may have been a jump to another host mammal that was also probably sold in the Seafood market in Wuhan, most probably a pangolin (Manis javanica). Any virus can “spill over” or “jump species,” but to be infective for the new species, it needs to mutate. These mutations can occur in the old host, or new, or both. This spilling over cannot be predicted and thus, pre-emptive vaccine development is not the ultimate solution, even though timely diagnosis and isolation of the confirmed positive patients, and suspected exposed individuals are the characteristics for immediate containment of this pandemic. Molecular studies, evolutionary models, and phylogenetic analysis are indispensable to establish the evolutionary rate and the genetic variability, which in turn have significant inferences for progression of the disease as well as for development of the drugs and vaccine.

Virology

Coronaviruses are lipid-enveloped viruses with the non-segmented nucleic acid, which is of positive sense, single-stranded RNA genome, 26-32 kb long, thus, comes under Class IV of Baltimore classification of Animal Viruses, belonging to the Orthocoronavirinae subfamily and as the name suggests, it has distinctive “crown-like” spikes or “club-shaped” projections on their Outermost layer [Figure 1] and [Figure 2]. There are four coronavirus genera (alpha, beta, gamma, and delta) with human coronaviruses detected in alphacoronaviruses (NL63 and HCoV-229E) and beta coronaviruses (HCoV-OC43, HCoV-HKU1, MERS_CoV and SARS - CoV) genera. Coronavirus now has twenty-four species classified under it. They are almost spherical enveloped particles, but pleomorphic, 120–160 nm in diameter with a characteristic ‘fringe’ on the surface. These projections are each of 20 nm in length. The nucleocapsid is helical. Its structural proteins include: Membrane protein (M), envelope protein (E), Spike Protein (S), and nucleocapsid phosphoprotein; and the transcribed non-structural proteins include: orf1ab, ORF3a, ORF7a, ORF6, ORF8, and ORF10. A long, polycistronic messenger is produced from the positive stranded RNA genome that codes for a number of proteins, some of which are post translationally cleaved to smaller proteins.

The structural analysis of the virus has shown that the viral spike protein (protein S) has a role to play, firstly, after it binds to a cell receptor, it helps in the viral entry into the cell and secondly, membrane fusion. These are the two essential steps for viral infection and pathogenesis. Likewise, the N protein, which is involved in virion assembly is a structural protein, and plays a principal role in virus transcription and assembly efficiency. Mutation of these proteins could help determine two main characteristic features of the novel coronavirus, (i.e., i.) an enhanced ability to infect and ii.) increased pathogenicity than the bat-like SARS coronavirus but lower pathogenicity than the SARS coronavirus. In order to have a deeper understanding of the 2019-CoV zoonotic transmission and the severity of its spread, the genomic study needs to be done extensively.

Pathogenesis

The studies published by various researchers in China reason out that the principal destination of the virus are the epithelial cells of the lung. It has been described that the interhuman transmission of SARS - CoV occurs as the virus spikes bind with the cellular receptor which was discovered to be the angiotensin-converting enzyme II (ACE 2) receptor, The receptor binding domain receptors of SARS - CoV and Covid-19 show some similarity. This envelope spike glycoprotein (Protein S) is primarily responsible for its tropism to lung alveolar epithelium. The arrival of SARS - CoV into the host cell is known to be achieved by direct fusion of membranes of the virus and the plasma membrane of the epithelial cells. There is a crucial cleavage of proteins occurring of the S protein in SARS - CoV at position S2' causing the membrane fusion and therefore responsible for its infectivity. As virus enters the cell, there is a release of the viral genome in the cytosol and it is translated into structural and two polyproteins proteins, after which the replication of the virus begins.

After the formation of the envelope glycoproteins, they are introduced into the membrane of the Golgi apparatus or endoplasmic reticulum, and the nucleocapsid is formed by the union of genomic RNA and the nucleocapsid protein. These viral particles then encroach further to pullulate and form the Endoplasmic reticulum - Golgi Intermediate Compartment (ERGIC). These vesicles have the virus particles residing in them and they blend into the plasma membranes to emit the virus.
With the viral entry into the cells, the antigens are subjected to come in contact with the antigen presenting cells (APC), which is an essential component of the body’s immune system. Major Histocompatibility Complex (MHC) present the antigenic peptides that are recognized by the cytotoxic T lymphocytes, specific to the virus. MHC I molecules are relatively more responsible for the antigen presentation than the MHC II molecules.\[17\]

### Human Response

The virus specific T and B cells have a vital role in mediating the humoral and cellular immunity of the body after being stimulated by the event of antigen presentation. Like the other acute viral infections, the antibodies that are formed against the viral load have a classical profile of IgM and IgG production. The IgM antibodies typically appear in the second week after the onset of symptoms, whereas the IgG antibodies can last for over a few years. This indicates that the IgG antibodies are primarily specific to S and N proteins and protect from the reinfections.\[18\]

There is a significant reduction in the CD4 T cell count and CD8 T cell count in the peripheral blood of Covid 19 patients. The memory T cells can be found in an individual even in the absence of antigen, thereby allowing a recovered patient to produce Interferons and proliferate T cells, protectively in case there is a virus re-entry.\[19\] These findings are insightful for more predictive outcome of the patient’s condition and also very valuable so that the vaccine against Covid can be judiciously designed.

### Cytokine Storm

‘Cytokine storm’ is a general term applied to maladaptive cytokine release in response to infection and other stimuli. The pathogenesis is complex but includes loss of regulatory control of proinflammatory cytokine production, both at local and systemic levels. After multiple studies, it has been established that the common clinicopathology between the previous SARS and MERS and the current SARS infections is Acute Respiratory Distress. Among this conglomeration of various immune mechanisms is the “cytokine storm”. It is so peculiar and characteristically fulminant and fatal overload of cytokines in the bloodstream leads to multiorgan failure.\[20\] It is an aggressive and lethal inflammatory response with the release of various inflammatory mediators, mainly cytokines (IL-6, IL-8, IL-1β, granulocyte-macrophage colony-stimulating factor, and reactive oxygen species) and chemokines (such as CCL2, CCL-5, IFNγ-induced protein 10 (IP-10), and CCL3) by the stromal cells and chief inflammatory cells like the B and T lymphocytes, mast cells, monocytes, macrophages, and few other contributors like fibroblasts, keratinocytes, endothelial cells, glomerular mesangium, and tumor immune effector cells.\[21,22\] Just like the name suggests, the cytokine storm presents as an aggressive charge by the immune system, which not only contributes to the occurrence of extrapulmonary multiple-organ failure (ARDS) but also multiorgan failure because of the global hyper immune reaction leading to fatality in cases of SARS-CoV – 2 (due to multi-organ dysfunction and physiological deterioration), just like what was known in previous SARS and MERS.\[23\] Timely control of the cytokine storm in its early stage through such means as immunomodulators and cytokine antagonists, as well as the reduction of lung inflammatory cell infiltration, is the key to improving the treatment success rate and reducing the mortality rate of patients with COVID-19.

### Clinical Scenario

The incubation period for the symptoms to appear is around 5.2 days of Covid-19.\[24\] With a median of 14 days, the time frame for death to occur from the time of onset of symptoms varies from about 6 to 41 days.\[24\] There is a widespread resemblance in the symptoms between the previous beta coronavirus outbreaks and the present pandemic. Nevertheless, Covid - 19 manifested with some distinctive presentations that include the targeting of the airway, which is implicated by URTI symptoms like coughing,
sore throat, rhinorrhea, and sometimes, sneezing. Moreover, infected patients developed intestinal symptoms like diarrhea, which was not as prevalent as in MERS-CoV or SARS-CoV patients. Many individuals have presented with multiple opacities in the subpleural regions of their lungs, which are ground glass in appearance on chest radiograph and computed tomography. These opacities are most likely due to an increased inflammatory response induced by an extensive local and systemic hyper-immune reaction. Unfortunately, the treatment with inhalation interferon therapy hardly showed any clinical effect and was the cause of the development of progressive pulmonary opacities and worsening in a few cases [Figure 3].[28] The classical CT image shows bilateral ground-glass and consolidative opacities in the pulmonary parenchyma, very often, round morphologically distributed peripherally. Involvement of the lung with the periphery predominantly involved was also seen in previous SARS and MERS affected cases, and even the chest CT findings of ground glass and consolidative opacities back then, matched with the current SARS-CoV-2 infection.[29]

The pathogenesis of the current novel coronavirus still remains a mystery as there have been reports that this pneumonia-causing virus could be because of an abnormal phenomenon related to biochemistry of the hemoglobin. This inference was put forth, as a study was conducted in which 99 subjects infected with the virus were subjected to various Hb-related biochemical tests.[27] According to the recent studies, the multiorgan failure is identified to be a result of hypoxia and not the hyperimmune response alone. In this review, we want to highlight various studies across the globe, which enlighten us the various pathways ignited by the novel coronavirus. In a recent study, it was proposed that the structural and non-structural proteins of Covid-19 work as a team to attack the hem molecule, particularly the 1-beta chain of hemoglobin to dissociate the iron ion from the porphyrin. Therefore, it decreased the carrying capacity of Hb not only for oxygen, but also for carbon dioxide. The alveolar cells undergo intense inflammation as there is a massive reduction of oxygen–carbon dioxide gaseous exchange, resulting in a ground-glass like opaque lung parenchyma on radiology. Patients who are presenting with respiratory distress go through a stronger degree of disease progression. Diabetics and geriatric population have higher glycated hemoglobin, relatively. Glycated hemoglobin (HbA1c) is known to be reduced by the viral infection, which made the patients' blood sugar unstable. As the hem anabolic pathway was inhibited by the porphyrin complexes of the virus produced in the human body, it led to a wide range of diseases and infections. There are numerous theories regarding the origin of viruses, one of them is called the co-evolution theory. The co-evolution theory states that viruses can evolve from complexes of the nucleic acids and the protein. A recent study proposes that the virus could be bound to porphyrin, which would in turn explain the survival problems of the original virus.[27]

Another study in Italy[29] strongly suggests that even though patients are presenting with respiratory symptoms, cardiac issues are the main cause of mortality. Although pneumonia has been billed as the prominent feature of this illness, the point that researchers are making was that no one was dying due to hypoxemia. Late recognition of cardiac involvement and decompensation was common in the patients who died. Hemodynamic decompensation can be sudden or more gradual and subtle. Elevation in high sensitivity troponin is the best marker they have identified for cardiac involvement (also for mortality).

Based on observations in European and American countries, and from post-mortem reports showing pulmonary involvement in suspected cases of Covid-19, majority disclosed that it was pulmonary thrombosis, which was not a typical ARDS presentation; but more disturbing was the hypoxemia that did not respond to PEEP and only to high oxygen flow.[29]

We can summarize the above studies and understand that the key pathogenic molecular step of SARS-CoV2 is to attack the 1-beta chain of Hb and hunt the porphyrins dissociating the iron from it and releasing it into the circulation. Thus, Hb loses its oxygen-carrying capacity, depriving the major organs of oxygen. This explains why we see resistant hypoxia coupled with very rapid multi-organ failures. Moreover, the unbound iron radical causes a powerful oxidative damage to the lung parenchyma because of its toxicity. Free iron toxicity causes inflammation of alveolar macrophages, leading to characteristic changes in the CT scan. To compensate for this, there is an increased rate of synthesis of Hb that leads to high Hb levels. Along with this, the iron gets stored in the form of ferritin which explains the high levels of ferritin in patients too. Therefore, the cause of monocytosis is the need for excess macrophages to engulf the excess iron load.

Figure 3: Commonly identified symptoms of Covid-19 showing general similarities with that of other beta coronaviruses
And the cause of Lymphopenia is the WBCs’ differentiation favored toward monocytes line rather than lymphocytes line. This makes ferritin a bad prognostic marker (increased iron stores indicate that most of the Hb has lost its Oxygen carrying capacity). Also, this iron load and increased Hb production leads to increased blood viscosity with recurrent and diffuse micro and macro circulatory thrombosis, and that is why there are high levels of D-dimer in those patients. This explains the cause of sudden deterioration and death in some sporadic cases, which was proved by post-mortem examinations of ARDS victims.

Management Strategies

The virus has presented differently in different patients, ranged from being asymptomatic to unpredictable and fatal cytokine storm, though the target organ is the lung. There is no harm in believing that we might have to live with Covid-19 for years to come; so a better understanding of this virus along with the knowledge of the armamentarium to tackle the same should be a must not only for medical practitioners but also for everyone else.

i. Investigations of Suspects

As we know, the presentation of Covid - 19 is usually a respiratory one with viral etiology, thus, many differential diagnosis should be considered. Travel history and history of contact with patients is vital to rule out suspects. For the diagnosis of Covid - 19, a blood sample of 5 ml in a plain vacutainer, without any additive for serology, upper aerodigestive tract swabs (nose and throat), and lower respiratory tract samples (bronchoalveolar lavage or sputum) can be collected.

Real-time quantitative polymerase chain reaction (RT-qPCR) and high through-put sequencing are nucleic acid detection technologies, which are most commonly used for for SARS - CoV - 2. The identification method that can define a treatment modality is high through-put sequencing of the whole genome and virus blood culture, yet RT-PCR assay is used for confirmatory laboratory diagnosis for detection of the viral RNA as it is effective, straightforward, and the most common tool for detecting pathogenic viruses in respiratory secretions and blood. Depending on the sample type, number of clinical specimens collected and the protocol used, RT-PCR for the detection of SARS - CoV can only achieve a sensitivity of 50-79%. Since it cannot be ignored that false negatives, however miniscule, have severe consequences, clinicians propose chest CT and repeated RT-qPCR to be more significant [Table 1].

There has also been a development of rapid diagnostic testing kits where the serological testing shows positivity of IgG or IgM antibodies indicating the timeline of the infection and the current infectivity state. However, serological tests have not yet got approval even though they are rapid, easy to perform, and cost effective; molecular testing stays gold standard. The role of CT-Chest has been prodigious in the diagnosis and management of Covid-19 by evaluating the disease progression. Each of the five lung lobes are assessed for the degree of involvement and classified as none (0%), minimal (1–25%), mild (26–50%), moderate (51–75%), or severe (76–100%). No involvement corresponded to a lobe score of 0, minimal involvement to a lobe score of 1, mild involvement to a lobe score of 2, moderate involvement to a lobe score of 3, and severe involvement to a lobe score of 4. An overall lung “total severity score” was reached by summing the five lobe scores (total severity scores ranged from 0–20). The study conducted by Bernheim et al. showed that the frequency of CT findings is directly proportional to infection time course. CT findings are more frequent after a longer time from the onset of symptoms, which include bilateral and peripheral disease, linear opacities, consolidation, total lung involvement, crazy paving pattern, and the reverse halo sign. Other rare radiological imaging presentations of this viral pneumonia are tree-in-bud opacities and other small nodules, bronchial wall thickening, and bronchial mucus plugs. Jin et al. classified CT findings of COVID-19 to five temporal stages as ultra-early, early, rapid progression, consolidation, and dissipation stages. Many other investigative efforts by the researchers have put forth mere imaging patterns like ground-glass opacities and peripheral consolidations with absence of pleural effusion and lymphadenopathy on chest-CT. Thus, it can be used on a larger scale to help identify and investigate suspected or confirmed Covid – 19 cases, though it sometimes overlaps with findings of other viral pneumonias.

The progression of this novel viral disease has been immensely unpredictable, but many studies have been conducted and one such investigative effort by Liu et al. have introduced a promising predictive factor—the neutrophil-to-lymphocyte ratio (NLR) which, along with age can predict who are more prone to develop critical illness. Thus, elderly patients and those having an NLR ≥3.13 should be prepared to be shifted to the intensive care as a precautionary measure.

With the COVID-19 outbreak and the upgrowing worldwide pandemic with the catastrophic mortality, the inclusive understanding and characterization of the diagnostic imaging findings, the variable criteria, and the chest imaging lineaments are crucial for proper patient management and treatment.

ii. Treatment Strategies

Until recently, it was well accepted that ARDS is the main cause of mortality, but recent studies have shown that it is only a subset. This viral disorder though self-limiting in 90% of the individuals,
required the administration of antiviral within 48 hours in order to curb the viral multiplication. Though multi-organ failure is known to be caused by the hyperimmune response, studies are under evaluation that suggest that it could be because of hypoxia as the viral proteins bind with porphyrin of the hem in the hemoglobin thus, irreversibly reducing the oxygen-carrying capacity of the red blood cells, in turn leading to severe inflammation of the lungs, causing ARDS. According to ARDS protocol, hyperbaric oxygen has shown significance in curbing the cytokenic storm syndrome. An evidence of hyperinflammation such as raised serum levels of ESR, CRP, or ferritin are definite indications to actively introduce anti-inflammatory drugs like indomethacin and hydroxychloroquine. Raised levels of fibrinogen and d-dimer have been dealt with administrations of anticoagulants like heparin and nafamostat. Clinical trials also suggest the use of plasma therapy in which convalescent plasma containing IgG and IgM antibodies directed against the novel coronavirus are infused, and many patients have shown recovery without any adverse reactions to the transfusion. Fluid balance cannot be ignored as 10-35% have gone into renal failure, which was the cause of mortality in few cases. Few researchers comment the need for factor Xa monitoring and full heparinization to approach the pro-thrombotic tendency. Many drug regimens, like the use of adenosine analogs, which block the viral RNA synthesis by targeting the RNA-dependent RNA polymerase, ACE inhibitors to block the entry of SARS-CoV-2 by inhibiting the binding of S protein with ACE2 receptor, broad spectrum antiviral treatments like Ribavirin, Lopinavir, Nelfinavir have shown potential in eradicating the virus, but further studies are on-going to state universal treatments guidelines.

Currently, there is no substantiated treatment for SARS-CoV-2 identified, and mainly supportive and symptomatic treatment are given. The pathogenesis of the virus is very important to know which class of antiviral drugs are to be administered. Few researchers have attempted to tackle the progression in order to curb the lethal cytokenic storm by drugs like TNF- blockers, interleukin family antagonists, corticosteroid therapies, administration of IFN-λ, IFN inhibitors, and so on. Adenosine and nucleoside analogs are prescribed, but they have not been universally helpful. A candidate for the virus may be the spike protein. There are currently over 30 vaccines under production in different stages of clinical trial.

End Note

In spite of the medical advances over the past 18 years, the threat of coronaviruses remains unparalleled. The ultimatum for the near future is not only to acquaint with the pathophysiology of SARS-CoV-2, but also to introduce new advancements to form a deeper understanding during a future zoonotic, coronavirus outbreak. In the midst of the hide-and-seek challenge between the virus and its variable spectrum of progression, it is important to discover in detail the accurate and exact pathogenesis as this is essential in establishing new treatment protocols, form better targeted therapies, and get closer to developing a vaccine, and getting the maximum of its effectiveness. This review intends to bring forth the theories about the pathogenesis of Covid – 19, which will help the health professionals initiate further research in order to discover more about coronaviruses so that history doesn't repeat itself in the following decades. In summary, the new-type coronavirus infection causes an inflammatory cytokine storm in patients. The cytokine storm leads to ARDS or extrapulmonary multiple-organ failure and is an important factor that causes COVID-19 exacerbation or even death. Timely control of the cytokine storm in its early stage through such means as immunomodulators and cytokine antagonists, as well as the reduction of lung inflammatory cell infiltration, is the key to improving the treatment success rate and reducing the mortality rate of patients with COVID-19.

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Conflicts of interest

There are no conflicts of interest.

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