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COVID-19: Understanding its virulence and pathophysiology

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Abstract
Coronavirus disease earlier known as "Pneumonia of unkown etiology" has emerged as a 6th public health emergency of international concern. It is a new public crises threatening the world with its infectious nature owing to its rapid transmission. COVID-19 is the disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus structurally similar to its ancestors by SARS-CoV and the Middle East respiratory syndrome coronavirus (MERS-CoV) responsible for the viral epidemics in 21st century. COVID-19 virus is reportedly much more virulent than either the SARS or MERS virus. Its ability to cause serious consequence contributing to the nature of the virus, its pathogenicity and host immune factors.
Based on the published evidence this review aims at providing comprehensive summary on microbiology of SARS-CoV-2, virulence, immune pathogenesis, associated pathophysiology. It's an attempt to aid the health care workers in containing the pandemic, offering novel insights, potential therapeutic targets for combating the SARS-CoV-2 infection and provide reference for future studies.

Keywords: Covid -19, SARS-CoV-2, spike glycoprotein, Coronavirus

1. Introduction
Coronavirus is one of the major pathogens belonging to family of viruses, primarily targeting the human respiratory system. In past two decades, viral epidemics associated with coronaviruses (CoVs) such as, severe acute respiratory syndrome (SARS-CoV) and the Middle East respiratory syndrome (MERS-CoV) have presented a grave threat to public health [1, 2].
One such virus belonging to the family Coronaviridae was found to be accountable for a sequence of unexplained cases of pneumonia reported in Wuhan, China in December, 2019. The first few cases were classified as "pneumonia of unknown etiology", as the causative agent was unidentified. However after detailed investigation conducted by PRC Centres for Disease Control (CDC), experts confirmed that the pneumonia was caused by a novel coronavirus [2, 3]. On 11th February, 2020, the World Health Organisation (WHO) Director-General, Dr. Tedros Adhanom Ghebreyesus, officially named the disease caused by this virus, Coronavirus disease 2019 (COVID -19) [4].
The International Virus Classification Commission reference name for the virus is severe acute coronavirus respiratory syndrome (SARS-CoV-2) [5].
Within a short period of time the new virus was reported to be spreading relentlessly in mainland China as well as globally. The WHO confirmed the outbreak of COVID-19 as a Public Health Emergency of International Concern (PHEIC) at its meeting on 30th January, 2020 in accordance with the International Health Regulations (IHR, 2005) [6].
With exponential growth of infected individuals and alarming level of inaction, COVID-19 infection was declared as a pandemic on 11th March, 2020.
On 7th July, 2020, WHO declared the potential for airborne spread of COVID-19. As of 31st August, 2020, 216 countries have been afflicted all around the world. COVID-19 remains severe, incessant disease expanding worldwide with 25,118,689 cases [7].
Given the impact it has on public health, the research community has been working on developing countermeasures to treat this threat. In order to aid healthcare workers around the world to better deal with the SARS-CoV-2, the current review aims at providing insights on Virulence, Microbiology and Pathophysiology of the COVID-19 virus.

2. Microbiology and Virulence of COVID-19
Coronavirus are positive-sense single-stranded RNA (+ssRNA) virus, named for their crown like appearance, owing to the presence of spike glycoproteins on the envelope surface. They are zoonotic in nature and cause symptoms that range from common cold to more severe respiratory, enteric, hepatic and neurological symptoms [8, 9].
They belong to the subfamily Orthocoronavirinae of the Coronaviridae family of order Nidovirales, classified into four genera of CoVs: Alphacoronavirus (alphaCoV), Betacoronavirus (betaCoV), Deltacoronavirus (deltaCoV),

and Gammacoronavirus (gammaCoV). Till date, seven human coronaviruses (HCoVs) have been identified, α coronavirus (HCoV-229E and NL63) and β coronavirus (MERS-CoV, SARS-CoV, SARS-CoV2, HCoV-OC43 and HCoV-HKU1) generae which cause infections in humans [10, 11].

SARS-CoV-2 has a round or elliptical shape and is often pleomorphic with a diameter of approximately 60-140 nm. It is sensitive to ultraviolet rays and heat. Moreover, these viruses can be effectively deactivated by lipid solvents including ether (75%), ethanol, peroxyacetic acid, chlorine based disinfectant, chlorhexidine [12]. SARS-CoV-2 remains viable in aerosols for a duration of 3 hours. It is noticed to be stable on plastic and stainless steel up to 72 hours, followed by decrease in its viral titre [13].

The genome of the new SARS-CoV-2, showed 89% nucleotide homology with bat SARS-like-CoVZXC21 and 82% with that of human SARS-CoV. And hence, the name SARS-CoV-2. This evidence strongly supports the origin of SARS-CoV-2 from bats, although the intermediate hosts of SARS-CoV-2 is still undetermined as mutation in the original strain could have directly triggered virulence towards humans. Therefore there is still uncertainty regarding existence of this intermediary [12]. In another study, novel COVID-19 complete genome was compared with other related corona virus to provoke the mutation and the gaps. By analysing the series compatibility of the protein sequences under study, it was confirmed that there is a match between strains of COVID-19 with obvious differences with respect to other coronavirus family species. This suggests that COVID-19 originated from mutations which occurred in coronavirus family. More clearly, new mutations, especially in glycoproteins, may be created as there is high probability of causing changes in it [14].

The coronavirus genome encrypts four major structural proteins: nucleocapsid (N) protein, membrane (M) protein, envelope (E) protein and the spike (S) protein. Coronavirus mainly recognizes the corresponding receptor on the target cell through this S glycoprotein which promotes host attachment and fusion of the viral and cellular membranes for entry. The S glycoprotein is made up of a short intracellular tail, a transmembrane anchor, and a large ectodomain consisting a receptor binding S1 subunit and a membrane-fusing S2 subunit [15]. The cellular receptor identified in SARS- CoV-2 is Angiotension-converting enzyme 2 (ACE2) same as SARS-CoV due to their similarity in amino acid sequence of the tentative receptor-binding domain (RBD). ACE2 receptor is type I transmembrane metallo-carboxypeptidase mainly expressed in lung, intestine, kidney and blood vessels. It is also found in oral mucosa and salivary epithelium, predisposing the host to easy viral entry [16, 17].

Analysis of the structure model shows that SARS-CoV-2 binds ACE2 above 10 times the affinity of SARS-CoV. This threshold is higher than that required for viral infection. These results explain the more rapid transmission ability of the SARS-CoV-2 in humans than SARS-CoV, with number of confirmed COVID-19 much higher than people with SARS-CoV infection. Considering the higher affinity of SARS-CoV-2 to ACE2, soluble ACE2 might be a probable candidate for COVID-19 treatment. Also, S is the main antigen present at the viral surface and is the target of neutralising antibodies during infection thus making it a focus of vaccine design [18, 19].

Therefore the mechanism about whether binding of S-protein to ACE2 causes SARS-CoV-2 infection in human, how strong the interaction is for risk of human transmission, and how SARS-CoV-2 causes pathological mechanisms of organs damage remains unknown, which necessitates further research.

However, an Indian study reported two novel mutations in the Spike protein sequence of Gujarat's isolate. These mutations are based on a comparison of the original Wuhan sequence.

Gujarat isolates revealed the two mutations (Q271R) and (D614G). Both these mutations have been found to lie near the RBD in S1 domain of spike protein thus pointing towards a possibility of modulation of the receptor binding activity of S1 domain. Out of these two Q271R has been found to affect the secondary structure of the S1 domain. Since both of these mutations lie near the receptor binding domain, they may influence the spike receptor interactions by changing the conformation of the spike protein S1 domain. These mutations having appeared after initial Wuhan outbreak, might have evolutionary advantage over original strain. Mutations in S protein might either lead to better binding to host cell receptor and increased virulence or reduced binding to host cell receptor and thus reduced virulence with adaptation to the new environment. This might eventually generate antibody escape mutants and produce variants capable of binding to alternate receptors and sites, thereby escalating tissue tropism [20].

3. Pathophysiology

3.1 Coronavirus entry and replication in host cells

S spike glycoprotein in coronavirus envelope is a significant contributing factor for viral entry into the host cell [21]. After binding of S spike protein to ACE2 receptor the entry of coronavirus into cell was primarily found to be accomplished by direct membrane fusion between the virus and cellular membranes [22]. Moreover, Belouzard *et al*. established that the irreversible conformational changes due to proteolytic cleavage at (S2') facilitated the membrane fusion and viral infectivity [23]. A contrasting feature in SARS-CoV-2 in comparison to SARS-CoV as SARS-COV S spike is incorporated into assembly without being subjected to cleavage during biosynthesis [24]. The coronavirus spike is unusual among viruses because a range of different proteases including trypsin, cathepsins, elastase, the host type 2 transmembrane serine protease (TMPRSS2) (36), and plasmin, which can cleave and activate it [25]. The unique feature of SARS-CoV-2 when compared to other coronaviruses is the existence of furin cleavage [26]. Although the S1/S2 site was also subjected to cleavage by other proteases such as transmembrane protease serine 2 (TMPRSS2) and cathepsin, the ubiquitous expression of furin likely makes this virus very pathogenic [25].

On entry into the cells, viral RNA genome is released into the cytoplasm and begins to replicate after it is translated [11].

3.2 Humoral and cellular immunity

Once the virus enters the cells, the viral antigen is presented to antigen presenting cells (APC's). This consequently stimulates the body's humoral and cellular immunity. This is mediated by virus-specific B and T cells similar to the ones seen in common viral infection. Coronavirus specific IgM antibodies produced as first line of defence disappears by

the end of week 12, while the IgG antibody can lasts for a longer period. The difference in the type of humoral cells is found to be a significant determinant in age wise severity of COVID disease [27].

Cellular immunity in latest report demonstrates remarkable reduction in the number of CD4+ and CD8+ T cells in the peripheral blood of SARS-CoV-2-infected patients. Contrary to that its status is excessive activation, as evinced by high quantities of HLA-DR (CD4 3.47%) and CD38 (CD8 39.4%) double-positive fractions [28].

3.3 Immune responses in children versus adults

Adults have shown to be more susceptible to SARS-CoV with different outcomes ranging from asymptomatic to severe disease, and death. Children can also be infected by SARS-CoV-2, but most paediatric cases presented with mild SARS-CoV-2 infection [29]. Children are otherwise more vulnerable to other infections; thus, the question arises — why are children less susceptible to COVID-19 when compared to adults? In this line a couple of hypothesis can be considered.

Innate immune system of children can be a key factor to understanding protection mechanism in children. The immune readiness of children to any novel pathogen like SARS-CoV-2 might be contributed to several factors. First, in the initial phases of infection, natural antibodies, mostly of IgM isotype play an important role. These natural antibodies generated independent of former antigen have a broad reactivity and a variable affinity, which helps in containing the infection during the two weeks. It is essential for production of high-affinity antibodies and memory B cells (MBCs) that will clear the virus and prevent reinfection. In humans, natural antibodies are produced by immature IgM MBCs, these MBCs are produced in abundance in children. Secondly, children have the ability to rapidly generate natural antibodies with broad reactivity that have not yet been selected and shaped by the reaction to common environmental pathogens [30, 31, 32]. Whereas, in elderly people, majority of MBCs are highly mutated and specific, recognise their targets but appear incapable of adaptation to new antigens [31].

Unlike children, adult patients express supressed adaptive immunity and dysfunctional over-active innate immune response in case of severe infections. Presentation of normal leucocyte count in children with covid-19 is indicative of an appropriate immune response. These could be related to immune-senescence in elderly. Although decreased killing capacity exhibited by T cells at early stage after birth may explain susceptibility to SARS-CoV-2 in infants. As age advances, continuous antigen stimulation along with Thymus involution result in a shift in T cell subset distribution from naïve T cells to central memory T cells, effector T cells and effector memory T cells [33,34].

There have also been suggestions that the ACE2 receptor which binds to SARS-CoV-2 S protein is less mature at a younger age and shows abundant expression in well-differentiated ciliated epithelial cells [35]. Additionally gender also may affect expression of ACE2 receptor owing to location of ACE2 gene on the X-chromosome. Leading to high circulating ACE2 levels in men than in women [36].

Another possiblity is the simultaneous presence of other viruses in the mucosa of lungs and airways which is common in young children. They may eventually compete with SARS-CoV-2 virus, limiting its growth [37, 38].

3.4 Cytokine storm in COVID-19:

Acute Respiratory Distress Syndrome (ARDS) is a common immune-pathological event and main cause of death in COVID-19. Cytokine storm is an unrestrained systemic inflammatory response occurring subsequent to release of enormous amounts of pro-inflammatory cytokines and chemokines. It's one of the most important mechanism seen in ARDS that elicits a violent attack by the immune system to the body, which may result in multiple organ failure, leading to death in severe cases [28].

SARS-CoV-2 is transmitted predominantly through respiratory droplet, contact and potentially in fecal-oral [13]. The average incubation period ranges from 1 to 14 days [39]. On transmission primary viral replication is supposed to occur in mucosal epithelium of upper respiratory tract (nasal cavity and pharynx), with added multiplication in lower respiratory tract and gastrointestinal mucosa, giving rise to a mild viraemia [40].

COVID-19 can be divided into three phases that correspond to different clinical stages of the disease:

Stage 1: Asymptomatic state (initial 1–2 days of infection)

At the inception of infection the inhaled virus SARS-CoV-2 is more likely to bind to epithelial cells in the nasal cavity and starts duplicating. In vitro data with SARS-CoV reveals that the ciliated cells are primary cells which are infected in the airway [41]. Single-cell RNA shows low degree of ACE2 expression in conducting airway cells with no apparent preference for a cell type [42]. There is local multiplication of the virus but minimal innate immune response driven by monocyte/macrophage infiltration. At this stage this virus can be detected by nasal swabs which might be more sensitive than throat swabs. These individuals are infectious despite the low viral burden.

Stage 2: Upper airway and conducting airway response (next few days)

As the infection progresses the virus travels and migrates through the airways along the respiratory tract, eliciting a more robust innate immune response. Nasal swab or sputum will contain the virus (SARS-CoV-2) as well as early markers of the innate immune response. The disease COVID-19 is clinically expressed at this time. The level of C-X-C motif chemokine 10 (CXCL10) can be predictive of the subsequent clinical course [43]. CXCL10 is an interferon responsive gene which has been reported to be useful as a disease marker in SARS [43, 44, 45]. Determining the host innate immune response might improve predictions on the subsequent course of the disease and require more intensive monitoring. For about 80% of the infected patients, the disease will be mild and mostly restricted to the upper and conducting airways [45]. These individuals may be monitored at home with conservative symptomatic therapy.

Stage 3: Hypoxia, ground glass infiltrates, and progression to ARDS

The disease progresses to stage 3 in approximately 20% of infected patients who develop pulmonary infiltrates, some of which will lead to very serious outcomes. Initial estimates of the fatality rate are around 2%, but this varies with age [46]. The virus is now entering the gas exchange units of the lung and infects alveolar type II cells. Both SARS-CoV and influenza specially infiltrate type II cells

compared to type I cells [47,48]. The apical cilia on airway cells and microvilli on type II cells maybe vital for facilitating viral entry.

The infected alveolar units tend to be peripheral and subpleural [49, 50]. SARS-CoV migrates within type II cells, releasing huge amount of viral particles, and thereby causing the cells to die by undergoing apoptosis [45]. The end result is likely to be a self-replicating pulmonary toxin as the viral particles released infect the cells of type II in adjacent units. Type II cells are most likely lost in parts of the lungs, and secondary pathway for epithelial regeneration will be triggered. Normally, type II cells are the precursor cells for type I cells [51, 52].

Diffuse alveolar damage with fibrin rich hyaline membranes and a few multinucleated giant cells are the pathological consequences of SARS and COVID-19 [28, 53]. The abnormal wound healing can result in more serious scarring and fibrosis than other types of ARDS [28].

4. Clinical Presentation

SARS-CoV-2 virus primarily affects the respiratory system ranging from minimal to severe respiratory failure and multiple organ failure. Common clinical manifestations are similar to the symptoms of SARS-CoV and MERS-CoV infections including fever, non-productive dry cough, dyspnoea, fatigue, myalgia, normal or decreased leukocyte counts, and radiographic evidence of pneumonia [54]. Even in asymptomatic patient, characteristic pulmonary ground glass opacification can be seen on Computerized tomography (CT) scan. Additional non-classical symptoms presented by Covid-19 patients are isolated gastrointestinal symptoms, olfactory and/or gustatory dysfunctions [55, 56].

Complications of COVID-19 include impaired function of the heart, brain, lung, liver, kidney, and coagulation system.

Association of comorbidity with high prevalence of COVID-19 was recorded by the Chinese Novel Coronavirus Pneumonia Emergency Response Epidemiology Team. Most common comorbidities being hypertension, diabetes, cardiovascular disease and chronic respiratory disease [57].

5. Oral Manifestation

As saliva is being implicated as a reservoir for Sars-CoV-2 due to detection of its RNA in saliva before emergence of lung lesions, some oral manifestations have been reported. Most common oral signs reported are oral dryness, vesiculobullous lesions, aphthous-like lesions, dysgeusia and anosmia [58]. In about 3% of patients, anosmia or ageusia may be the only presenting symptom [59].

In another case report, a patient presented with apthous like lesions, burning sensation and tongue depapillation. Commissural chelitis is yet another symptom observed. Association of not only immunosuppression but stress has been associated with appearance of oral manifestations [60].

Speculations regarding 2019-nCoV causing acute sialadenitis have been made as 2019-nCoV binds to ACE2 receptors on the epithelium of salivary glands and as the virus can be detected in saliva [61].

Saliva is also being considered as a diagnostic tool in identification of individuals that have been infected with SARS-CoV-2 even if the infection was asymptomatic.

6. Conclusion

To sum up, COVID-19 is a serious bio-hazard that has plagued countries worldwide. The virus is highly contagious and transmitted through droplets, especially on close contact. It has posed a great threat to the global health care affecting all the strata of society. “Solidarity trials” for development of specific drugs and vaccines were initiated by WHO in 10 countries on March 2020. Currently phases I and II trials of Oxford vaccines ChAd0x1 are being conducted. Despite of the ongoing trials in several countries, the incidence of COVID disease is ever increasing. This makes it imperative to encourage the public in aiding the health care workers by adopting measures like social-distancing, home quarantine, work from home jobs to control the infection. This will help relieve the strain on health care system and to reduce mortality and morbidity of the COVID-19 disease.

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