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An Explication of Pandemic Public Health Emergency, Coronavirus Disease 2019 (COVID-19): A Review

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ABSTRACT

In December 2019 a series of acute atypical respiratory disease occurred in Wuhan, China. The first clusters of cases were identified in association with the South China Seafood Market. In subsequent investigations it was found to be a novel coronavirus. It is suggested to have zoonotic origin. On December 31st 2019, China notified the outbreak to the World Health Organization. During the New Year, the massive migration of Chinese fuelled the epidemic. Cases in other provinces of China and other countries (South Korea, Thailand and Japan in quick succession) were reported in people who were returning from Wuhan. On 11 February, on the basis of existing rules on taxonomy the virus was names as SARS-CoV-2. SARS-CoV-2 belongs to the family of coronaviruses. It is a positive-sense single-stranded RNA (+ssRNA) virus. It has a single linear RNA segment. On the same day WHO announced the new name for the disease i.e. Coronavirus disease 2019 (COVID-19). The WHO and the US Centers for Disease Control and Prevention (CDC) say it is primarily spread directly due to close contact between people through small droplets produced during coughing, sneezing or talking within a range of about 1-3 meters. It may even transmit through indirect contact via fomites. While there are concerns it may spread by feces, this risk is believed to be low. Soon, the number of cases started increasing exponentially and on March 12, 2020 WHO announced COVID-19 a pandemic. COVID-19 has been impacting a large number of people worldwide, being reported in approximately 200 countries and territories. It was identified that Angiotensin converting enzyme 2 (ACE2) act as a functional receptor for SARS-CoV-2. The pathophysiology of COVID-19 follows sex differences, age differences, race differences in as well as underlying disease conditions i.e. comorbidities aggravated the severity of this disease. The most common symptoms being reported are fever, dry cough or chest tightness, and dyspnoea. It is now widely recognized that respiratory symptoms of COVID-19 are extremely heterogeneous, ranging from minimal symptoms to significant hypoxia with ARDS. Diagnosis is done with the help of history, clinical signs and serological testing. Real-time reverse transcription polymerase chain reaction (rRT-PCR) is considered the standard method of testing. Several have been tested in clinical trials but none of them have been proven to be a definite therapy yet. The evolution of the current outbreak has seen extraordinary measures put in place to control transmission, including the 'shut-down' and 'quarantine'. Researchers are trying to develop a vaccine against SARS-CoV-2 but at present, no vaccine is available. One should strictly follow all the preventive measures as directed by WHO and CDC and along with this, one should boost up its natural immunity to lessen the chances of getting infection.

Keywords: SARS-COV-2, COVID-19, ACE2.

1 Introduction

Health authorities identified a series of acute atypical respiratory disease occurred in Wuhan, China with unknown etiology during December 2019, which rapidly spread from Wuhan to other areas. In subsequent investigations, it was discovered that a novel coronavirus was responsible for this. The outbreak of SARS-CoV-2 was considered to have originally started via a zoonotic transmission associated with the seafood market, later it was recognized that human to human transmission played a major role in the subsequent outbreak caused by this virus (Li *et al.,* 2020). Initially WHO named the virus causing this illness as Novel Coronavirus, but on 11 February 2020, the International Committee on Taxonomy of Viruses (ICTV) identified 2019-nCoV as a strain of severe acute respiratory syndrome-related coronavirus and named as SARS-CoV-2, because according to existing rules that are based on five conserved sequences of nucleic acids that determine hierarchical relationships among coronaviruses ,the differences between what was then called 2019-nCoV and the virus strain from the 2003 SARS outbreak were not sufficient enough to make novel corona virus and SARS-CoV separate viral species. On the same day i.e. February 11, 2020 WHO announced a name for the new coronavirus disease: COVID- 19 and on March 12, 2020 a pandemic. COVID-19 has been impacting a large number of people worldwide, being reported in approximately 200 countries and territories (Zheng *et al.,* 2020; Zhang *et. al.,* 2020). SARS-CoV-2 virus primarily affects the respiratory system, although other organ systems are also involved. It is now widely recognized that respiratory symptoms of COVID-19 are extremely heterogeneous, ranging from minimal symptoms to significant hypoxia with ARDS (Yuki *et.* al., 2020). Current medical management is largely supportive with no targeted therapy available. Several drugs including lopinavir-ritonavir, remdesivir, hydroxychloroquine, and azithromycin have been tested in clinical trials (Cao *et. al*., 2020; Gautret *et. al.,* 2020; Zhou *et. al*., 2020) but none of them have been proven to be a definite therapy as yet. The evolution of the current outbreak has seen extraordinary measures put in place to control transmission, including the 'shut-down' and 'quarantine'. Preventive measures play a major role in prevention of this disease. Researchers are trying to develop a vaccine against SARS-CoV-2 but at present, no vaccine is available.

2 Origin and spread

In early December 2019, health authorities identified at Wuhan. The first clusters of cases were reported at the South China Seafood Market, where a large range of live or freshly slaughtered animals put on sale including poultry, bats, and snakes (Lake, 2020). On December 31st 2019, China notified the outbreak to the World Health Organization ("19" in COVID-19 represents its notifying year i.e. 2019) thereafter on 1st January the Huanan sea food market was closed. The number of cases started increasing exponentially, many of which did not have exposure to the live animal market, suggestive of the fact that human-to-human transmission was occurring (Huang *et al.,* 2020) which got confirmed later on (Chen *et al.,* 2020). The first fatal case was reported on 11th Jan 2020 (WHO, 2020). During the New Year, the massive migration of Chinese fuelled the epidemic. In China, Cases continued to increase exponentially and modelling studies reported an epidemic doubling time of 1.8 d (Li, 2020). Cases in other provinces of China and other countries (South Korea, Thailand and Japan in quick succession) were reported in people who were returning from Wuhan. Soon the city was locked down by government. Due to its ability to transmit from human to human by direct and indirect methods, soon cases started coming up in various countries like Italy, Europe, South Korea, India and other countries. Within these countries also the cases started increasing exponentially. On March 12, 2020 WHO announced COVID-19 a pandemic and disease of major public health concern with expected incubation period of COVID-19 is 2-14 days (WHO, 2020).

3 Virology

The SARS-CoV-2 belongs to the family of coronaviruses. It is a positive-sense single-stranded RNA (+ssRNA) virus. It has a single linear RNA segment. Coronaviruses are a broad family of viruses. Illnesses caused by coronaviruses ranges from the common cold to more severe diseases such as Middle East respiratory syndrome (MERS) and SARS. It is the seventh known coronavirus to infect people, after 229E, NL63, OC43, HKU1, MERS-CoV, and SARS-CoV (Zhu *et al.*, 2020) SARS-CoV-2 is closely related to the original SARS-CoV. Like the SARS-related coronavirus strain involved in the 2003 SARS outbreak, SARS-CoV-2 is a member of the subgenus Sarbecovirus (beta-CoV lineage B) (WHO, 2020). The length of RNA sequence of SARS-CoV-2 is approximately 30,000 bases. The SARS-CoV-2 is unique among known betacoronaviruses in its incorporation of a polybasic cleavage site, a characteristic known to increase pathogenicity and transmissibility in other viruses (Walls *et al.*, 2020; Coutard *et al.*, 2020). On 11 February 2020, the International Committee on Taxonomy of Viruses (ICTV) identified 2019-nCoV as a strain of Severe acute respiratory syndrome-related coronavirus and named it SARS-CoV-2 because according to existing rules that are based on five conserved sequences of nucleic acids that determine hierarchical relationships among coronaviruses, the differences between what was then called 2019-nCoV and the virus strain from the 2003 SARS outbreak were not sufficient enough to make novel corona virus and SARS-CoV separate viral species (Gorbalenya *et al.*, 2020). Diameter of SARS-CoV-2 virion is approximately 50–200 nanometres (Chen *et al.*, 2020). The SARS-CoV-2 has four structural proteins, known as the S (spike), E (envelope), M (membrane), and N (nucleocapsid) proteins like other coronaviruses. The S, E, and M proteins together create the viral envelope of the SARS-CoV-2 and N protein holds the RNA genome. The spike protein is responsible for allowing the virus to attach and fuse with the membrane of a host cell (Wu *et al.*, 2020). Protein modeling experiments on the spike protein of the virus suggested that SARS-CoV-2 has affinity to bind with the receptor angiotensin converting enzyme 2 (ACE2) on human cells and thereby enter inside the body (Chen *et. al.*, 2020; Letko *et al.*, 2020; Letko and Munster, 2020; Xu *et* al., 2020). For entry of SARS-CoV-2, initial spike protein priming by transmembrane protease, serine 2 (TMPRSS2) is essential (Hoffman *et al.*, 2020). After attachment of SARS-CoV-2 virion to a target cell, the spike protein of the virus is cleaved by cell's protease TMPRSS2 thereby exposing a fusion peptide. The RNA of the virion is then released into the cell and the cell is forced to produce and disseminate copies of the virus, which infect more cells (Hoffman *et. al.*, 2020; Ou *et. al.*, 2020).

4 Transmission

During this pandemic, human-to-human transmission of SARS-CoV-2 has been confirmed (Chan *et al.*, 2020). The WHO and the US Centers for Disease Control and Prevention (CDC) said, it is primarily spreading directly due to close contact between people through small droplets produced during coughing, sneezing or talking within a range of about 1-3 meters. Those released droplets can be possible inhaled into the lungs by the people who are nearby. Even some medical procedures such as intubation and cardiopulmonary resuscitation (CPR) may result in an airborne spread by causing respiratory secretions to be aerosolized. The serial interval for COVID-19 virus is estimated to be 5-6 days (WHO, 2020; CDC, 2020).

It may even transmit through indirect contact via fomites, when a person sneezes or coughs the droplets fall on the nearby surfaces and it may get transmitted when someone touches that contaminated surface and then touches his eyes, nose or mouth. The virus survives for hours to days on surfaces. Specifically, the virus was found to be viable for one day on cardboard, for up to three days on plastic and

stainless steel and for up to four hours on copper (van Doremalen *et al.*, 2020). This however, varies based on the humidity and temperature.

Viral RNA has also been found in stool samples from infected people (Holshue *et al.*, 2020). There are concerns it may spread by feces, the risk of is transmission by faeces is believed to be low (WHO, 2020). There is some evidence of human-to-animal transmission of SARS-CoV-2 also. In Europe, some house cats living with COVID-19 positive owners have also tested positive for SARS-CoV-2 infection (OIE, 2020). Recently, a tiger at the Bronx Zoo was found to be infected with SARS-CoV-2 (Goldstein and Joseph, 2020).

It has been indicated that the pharynx reaches peak viral load approximately four days after infection (Wölfel *et al.,* 2020; Kupferschmidt, 2020). The virus is most contagious when people are symptomatic, though spread is also possible before symptoms emerge and from those asymptomatic people who never developed symptoms. On 1 February 2020, the WHO indicated that transmission from asymptomatic cases is likely not a major cause of transmission (WHO, 2020). However, an epidemiological model of the beginning of the outbreak in Wuhan, China suggested that among documented infections pre-symptomatic shedding of virus may be typical and people with subclinical disease may have served as major source of infection to majority of infected people (Li *et al.*, 2020).

5 Reservoir

The anamnesis of first known infection from the SARS-CoV-2 strain, discovered in Wuhan, China (Zhou *et al.*, 2020) indicated, original source of viral transmission to humans as well as whether the viral strain became pathogenic before or after the spillover event is imprecise till now (van Doremalen *et al.,* 2020). The first cluster of cases were identified in association with the South China Seafood Market, a 'wet' market at which a large range of live or freshly slaughtered animals were sold including poultry, bats, and snakes (Lake, 2020). It has been suggested that the strain might have originated from the market itself (Wölfel *et al.,* 2020). However, other research indicates that visitors may have introduced the virus to the market, which then facilitated rapid expansion of the infections due to close contact of people there (Cyranoski, 2020). Initially bats were considered the most likely natural reservoir of SARS-CoV-2 (WHO). Samples were taken from *Rhinolophus sinicus* and analysis of some viral nucleic acid sequences showed a resemblance of 80% to SARS-CoV-2 (Benvenuto, *et al.*, 2020). A study showed that viral nucleic acid sequence in a sample taken from *Rhinolophus affinis*, collected in Yunnan province and designated RaTG13, has a 96% resemblance to SARS-CoV-2 (Zhou *et* al., 2020). In SARS outbreak also many SARS- like bat coronaviruses were discovered, mostly originating from genus *Rhinolophus* only but there are some differences between the bat coronavirus and SARS-CoV-2 which suggest a possibility that humans were infected via an intermediate host (Cyranoski, 2020). The virus genome has a novel lineage for almost half of its genome, with no close genetic relationships to those of its subgenus, sarbecovirus (Paraskevis *et al.*, 2020). On 7 February 2020, researchers from Guangzhou announced that they had discovered a pangolin sample in which viral nucleic acid sequence is "99% identical" to SARS-CoV-2 (Cyranoski *et al.*, 2020). The results clarified that with a difference of only one amino acid, the receptor-binding domain of the S protein is almost identical to that of 2019-nCoV (Xiao *et al.*, 2020). Since pangolins are critically endangered hence they are protected under government law, but their trafficking for use in traditional Chinese medicine and to be eaten as luxury food remains common. So, it is a possibility that transmission of SARS-CoV-2 had occurred due to trade of pangolins across the countries. Geneticists and microbiologists in Texas have separately found the evidence that reassortment has taken place in coronaviruses which suggests the involvement of pangolins in the origin of SARS-CoV-2 (Wong *et* al., 2020). However, only at most 92% of homology has been seen in genome of pangolin coronaviruses

found till date and SARS-CoV-2, making them less similar than RaTG13 to SARS-CoV-2 (Zhang *et* al., 2020). This is inadequate to prove pangolins to be the intermediate host because in comparison to it, the SARS virus responsible for the 2003 outbreak shared 99.8% of its genome with a known civet coronavirus (Cyranoski *et al.*, 2020). Previously a metagenomic study was published in 2019 which revealed that among a sample of Sunda pangolins, SARS-CoV: causative agent of SARS outbreak, 2003, was the most widely distributed coronavirus (Liu *et al.*, 2019). It also strengthens the possibility that pangolins serve as an intermediate host in transfer of SARS-CoV-2.

6 Pathophysiology

The expected incubation period of COVID-19 is 2-14 days with hyperinflammation, renin-angiotensin-aldosterone system imbalance, and a particular form of vasculopathy, thrombotic microangiopathy, and intravascular coagulopathy. SARS-CoV-2 impairs innate and adaptive antiviral responses, triggers hyperinflammation, and deranges the renin-angiotensin-aldosterone system (RAAS), all culminating to promote detrimental hypercoagulability and immunothrombosis. The SARS-CoV-1 and SARS-CoV-2 share up to 85% genomic identity, and both utilize the same primary human host receptor, angiotensin converting enzyme 2 (ACE2), to enter target cells (Zhou *et al.*, 2020).

6.1 Receptor bounded entry

It was identified that Angiotensin converting enzyme 2 (ACE2) act as a functional receptor for SARS-CoV (Li *et.* al., 2003). Structural and functional analysis of SARS-CoV-2 showed that the spike for SARSCoV-2 also bound to ACE2 (Chen *et.* al., 2020; Letko *et. al.*, 2020). In a study it was found that ACE2 expression was high in lung, heart, ileum, kidney and bladder (Zou *et. al.*, 2020).

6.2 Respiratory System

It was found that ACE2 receptors were highly expressed on lung epithelial cells. Whether or not SARS CoV- 2 binds to an additional target is not clear and needs further investigation. Following the binding of SARSCoV-2 to the host protein, the spike protein undergoes protease cleavage (Ou *et. al.*, 2020). Earlier, a two-step sequential protease cleavage to activate spike protein of SARS-CoV and MERS-CoV was proposed as a model, consisting of cleavage at the S1/S2 cleavage site for priming and a cleavage for activation at the S'2 site, a position adjacent to a fusion peptide within the S2 subunit (Belouzard *et. al.*, 2009) but whether the cleavage in SARS-CoV-2 follows the same pattern is still to be elucidate. After the cleavage at the S1/S2 cleavage site, S1 and S2 subunits remain non-covalently bound and the distal S1 subunit contributes to the stabilization of the membrane anchored S2 subunit at the prefusion state (Walls *et. al.*, 2020). Subsequent cleavage at the S'2 site presumably activates the spike for membrane fusion via irreversible, conformational changes. The coronavirus spike is unusual among viruses because a range of different proteases can cleave and activate it (Belouzard *et. al.*, 2012). SARS-CoV-2 has some unique characteristics among coronaviruses like the existence of furin cleavage site ("RPPA" sequence) at the S1/S2 site. The S1/S2 site of SARSCoV-2 is entirely subjected to cleavage during biosynthesis in a drastic contrast to SARS-CoV spike, which is incorporated into assembly without cleavage (Walls *et* al., 2020). Although the S1/S2 site was also subjected to cleavage by other proteases such as transmembrane protease serine 2 (TMPRSS2) and cathepsin L, the ubiquitous expression of furin likely makes this virus very pathogenic (Hoffman *et. al.*, 2020; Ou *et. al.*, 2020).

6.3 Digestive system

The receptors for SARS-CoV-2 virus are also present in gastrointestinal tract. The ACE2 is abundantly expressed in endothelial cells and enterocytes of the small intestine as well as the glandular

cells of gastric, duodenal and rectal epithelium (Hamming *et* al., 2020; Gu *et* al., 2020) which is probably responsible for gastro-intestinal signs in humans.

6.4 Nervous system

The ACE2 is also expressed in the brain, it is particularly present in the brain stem and in the regions responsible for cardiovascular function regulation including paraventricular nucleus (PVN), subfornical organ, rostral ventrolateral medulla and nucleus of the tractus solitarius (NTS); expression of ACE2 was found in both neurones as well as glia. But we cannot exclude the possibility of non-ACE2 pathways for virus infection of neural cells this is because marked penetration of coronavirus into the liver which is an organ with lower levels of ACE 2 compared to the CNS, supports the assumption that the cell entry routes can vary and suggested that he neuroinvasive potential of SARS□CoV2 may play a role in the respiratory failure of COVID□19 patients (Li *et al.*, 2020).

6.5 Vascular system

Since, vascular endothelium actively expresses ACE2 hence SARS-CoV-2 is capable of directly infecting these cells (Nicin *et al.*, 2020). A recently published histopathology report showed endothelial cell infection and inflammation of endothelial cells i.e. endothelitis in 3 patients with COVID-19 (Varga *et* al., 2020). Along with direct cytopathic effects it causes hyperinflammation which leads to local and systemic endothelial injury and dysfunction, this results in increased vascular permeability, excess thrombin generation and inhibition of fibrinolysis (Frantzeskaki *et al.*, 2017). It is seen that plasma levels of TF and PAI-1 are significantly elevated in patients with non-specific ARDS as compared to non-ARDS patients, leading to a lung coagulopathy driven by increased thrombin generation as well as bronchoalveolar depression of fibrinolysis (Ozolina *et al.*, 2016; Glas *et al.*, 2013). Moreover, there is increased risk of severe and fatal COVID-19 in patients with co-morbidities such as hypertension, diabetes and obesity may in part be due to the underlying endothelial dysfunction, which is common in these conditions.

Overall, as hyperinflammation progresses systematically, it may culminate in widespread immunothrombosis, contributing to organ dysfunction. The hypercoagulable state may potentially be further enhanced by other clinical factors including hypoxemia which is secondary to ALI/ARDS, hyperthermia and/or hypovolemia which is secondary to gastrointestinal fluid loss and/or negative fluid balance in the ARDS treatment Protocol (Meyer *et al.*, 2013). There is disseminated intravascular coagulation (DIC) and leukoerythroblastic reaction seen in patients of COVID-19 (Mitra, *et al.*, 2020; Lillicrap *et al.*, 2020).

6.6 Renin-Angiotensin-Aldosterone-System (RAAS) Derangement in COVID-19

The RAAS dysfunction plays a significant role in the pathophysiology of general acute respiratory distress syndrome (ARDS) (Vrigkou *et al.*, 2017; Zhang and Baker, 2017). The ACE2, which is host cell receptor of SARS-CoV-2, is a major component of RAAS (Zhou *et al.*, 2020). Thus, we hypothesize that RAAS aberrations contribute significantly to the likelihood of developing severe COVID-19 symptoms (Sanchis-Gomar *et al.*, 2020). The ACE converts angiotensin I (AngI) to angiotensin II (AngII), while ACE2 converts AngII to angiotensin 1-7 (Ang1-7). Thereafter, AngII binds with AngII receptor type 1 (AT1) and exerts pro-oxidative, vasoconstrictive, pro-inflammatory and even fibrotic effects. In contrary, Ang1-7 binds to the Mas receptor (MasR) and mediates anti-oxidative, vasodilatory, and anti-inflammatory effects (Guan *et al.*, 2020). This suggests that the binding of SARS-CoV-2 to ACE2 attenuates ACE2 activity via internalization, thereby disturbing the ACE/ACE2 balance to a state which is predominated by high levels of AngII, which causes pulmonary vasoconstriction, oxidative,

inflammatory and fibrotic organ damage which is the ultimate cause behind progressing towards ALI/ARDS. The RAAS system is intrinsically linked to the coagulation cascade and may exacerbate the processes of immunothrombosis, further driving microthrombi formation in COVID-19. First, AngII induces PAI-1 expression by endothelial cells via the AT1 receptor, contributing to a PAI-1/tPA imbalance and a hypercoagulable state (Nakamura *et al.*, 2000; Vaughan *et al.*, 1995). This may explain why unresolved fibrin deposits are observed in the alveoli of patients with general ARDS, a feature that has also been observed in lungs of both SARS and COVID-19 victims (Yao *et al.*, 2020).

Secondly, ACE metabolizes bradykinin. Bradykinin can stimulate vasodilation and release of tissue plasminogen activator (tPA) from endothelial cells (Brown *et al.*, 1999). It can be assumed that an inflammatory response to COVID-19 would lead to increased bradykinin production (and hence increased tPA expression). However, elevated AngII would lead to increased aldosterone that may further enhance ACE activity, which converts bradykinin to inactive peptides, blunting the bradykinin-mediated tPA increase (Stoll, 2020). Based on observations of hypokalemia, a hyperaldosterone state is suspected in cases of severe COVID- 19 (Lippi and Plebani, 2020). Moreover, aldosterone has also been shown to directly increase PAI-1 expression, especially in renal tissue (Brown *et* al., 2000; Ma *et* al., 2006). Hence, while bradykinin (and hence tPA) may be increased, the increased ACE, AngII and aldosterone (and hence PAI-1) is likely to be of greater magnitude, leading to a decreased tPA to PAI-1 ratio, promoting hypofibrinolysis. In addition to the microthrombi, this imbalance may lead to poor resolution of alveolar lesions and explain the significant degree of fibrosis observed in COVID-19 patients (Yao *et* al., 2020). ACE inhibitors (ACEi) have been shown to improve endothelial function and have been suggested to be associated with less severe COVID- 19 disease (Sanchis-Gomar *et* al., 2020). This suggests that hyperinflammation and detrimental immunothrombosis may be central to the pathophysiology of COVID-19. Platelet hyper-reactivity, hypofibrinolysis, complements over-activation, hypercoagulability and RAAS derangement in the presence of underlying inflammatory-induced endothelial dysfunction likely lead to a state of COVID-induced coagulopathy.

7 Immunopathology

The COVID-19 is associated with CD4+ and CD8+ T-cell lymphopenia, which may result from a combination of virus-induced direct cytopathic effects, as well as enhanced T-cell apoptosis due to a dysregulated cytokine milieu (Wang *et. al.*, 2020; Tan *et. al.*, 2020). Furthermore, CD4+ T-lymphopenia may impair the adaptive antiviral response through inadequate T-cell help to virus-specific CD8+ cytotoxic T-cells and B-cells.

8 Sex differences

Investigation of data obtained from China and Italy, it becomes clear that males are approximately 64-71% of total deceased COVID-19 patients (China CDC; Italian National Institute of Health, 2020) as well as in Europe also, out of total people infected with COVID-19, 57% were men; out of those who were infected with COVID-19 who also died, 72% were men (WHO, 2020). It has largely been attributed to gender differences in some risk factors (Wenham *et al.*, 2020). However, immunobiological sex differences may also contribute. The *TLR7* gene is located on chromosome X, and escapes X chromosome inactivation, resulting in enhanced expression in females (Souyris *et al.*, 2020). more pronounced IFN-α release from cells is induced by TLR7 agonists in females (Berghöfer *et al.*, 2006) and following TLR7 agonism, type I IFN release is enhanced by estradiol (Seillet *et al.*, 2012). The Biallelic TLR7 expression and subsequent estradiol signaling may potentially render females less prone to the viral type I IFN antagonism, which may lead to a more substantial type I IFN response in early stages of

disease. This may potentially improve the initial antiviral response and prevent the subsequent aberrant hyperinflammation, in part explaining increased disease severity in males. However, this theory requires additional studies.

9 Age group differences

Usually infants and young children are at high risk for admission to hospitals due to respiratory tract infection with viruses as influenza virus and respiratory syncytial virus. In contrast, pediatric COVID-19 patients have relatively milder symptoms in general compared to elder patients. The exact reason for this difference between children and adults remains elusive. But because a recent report suggested that there is correlation between the amount of viral loads and the severity of COVID-19 (Liu *et* al., 2020) so it is a possibility that the children may have less virus loads even if they get COVID-19. In support of this a couple of hypotheses can be considered.

The first possibility is that the expression level of ACE2 may differ between adults and children. A previous study showed that ACE2 was more abundantly expressed on well-differentiated ciliated epithelial cells (Hamming *et al.*, 2004). Human lung and epithelial cells continue to develop following the birth hence ACE2 expression may be lower in pediatric population.

The second possibility is that children have a qualitatively different response to the SARS-CoV-2 virus in comparison to adults. With ageing, continuous antigen stimulation and thymic involution leads to a shift in T cell subset distribution from naïve T cells to central memory T cells, effector T cells and effector memory T cells (Saule *et al.*, 2006). This process is accompanied by the loss of expression of co-stimulatory molecules such as CD27 and CD28, with increased susceptibility to infections (Li *et* al., 2019). Whether the appearance of pathological T cells in adult patients with severe COVID-19 diseases is due to the compensation for this fundamental aging process or not is unclear. At the early stage after birth, CD4+ T cells are impaired in production of Th1 associated proinflammatory cytokines and shift toward Th2 (Connors *et al.*, 2016). CD8+ T cells reduced expression of cytotoxic and inflammatory mediators. However, less killing ability by T cells at early stage after birth may explain susceptibility to SARS-CoV-2 in infants. But the study comparing aged and young macaques infected with SARS-CoV showed that aged macaques had more robust proinflammatory responses with worse lung pathology than young ones (Smits *et* al., 2010).

A similar result was reported using aged and young mice infected with SARS-CoV (Roberts *et* al., 2007). So similarly, it is expected that pro-inflammatory responses make this disease severe in adults as compared to infants and ageing is associated with increasing proinflammatory cytokines that govern neutrophil functions and have been correlated with the severity of ARDS. Severe COVID-19 infection is characterized by a massive proinflammatory response or cytokine storm that results in ARDS and multi - organ dysfunction (MODS).

The third possibility is that the simultaneous presence of other viruses in the mucosa lungs and airways, common in young children, can let SARS-CoV-2 virus compete with them and limit its growth (Nickbakhsh *et* al., 2019). It is possible that a combination of these possibilities helps in understanding why children in general are less susceptible to severe COVID-19.

10 Racial differences

It has been observed that during the outbreak of COVID-19 in the US, a greater proportion of deaths occurred among African Americans (van Dorn *et al.*, 2020). This is supposed to be because of their sub-standard living which prevents African Americans to follow social distancing because of crowded housing. As well as most of the African Americans are involved in 'essential occupations' like health care

workers and public transit services so they have to go door to door for their work. Most of them have high prevalence of underlying disease conditions like diabetes, asthma, cardiovascular diseases etc. So they are more prone to the infection. However, to know the exact cause behind this, further studies are required (Adams *et al.*, 2020).

11 Underlying disease conditions

The pandemic, COVID-19 appears to be more severe in patients with underlying disease conditions like hypertension, cardiovascular disease, and diabetes (Guan *et* al., 2020). These disorders are associated with reduced baseline levels of ACE2 expression, which makes the people with these underlying disease conditions more susceptible to SARS-CoV-2 mediated ACE/ACE2 imbalance (Tikellis *et al.*, 2020).

12 Signs and symptoms

The clinical features of COVID-19 are varied, ranging from asymptomatic state to acute respiratory distress syndrome and multi organ dysfunction. In a subset of patients, by the end of the first week the disease can progress to pneumonia, respiratory failure and death. The most common symptoms being reported are fever, dry cough or chest tightness and dyspnoea. Thus, they are indistinguishable from other respiratory infections. Conjunctivitis, throat, headache, fatigue, myalgia, diarrhea and loss of sense of smell and taste have also been described (WHO, 2020). Most reported cases experience a mild illness course. In addition, headache, dizziness, generalized weakness; vomiting and diarrhea were observed in some patients (Rodríguez-Morales *et al.*, 2020). It is now widely recognized that respiratory symptoms of COVID-19 are extremely heterogeneous, ranging from minimal symptoms to significant hypoxia with ARDS (Yuki *et.* al., 2020). Usually without needing hospital treatment, most people (about 80%) recover from this disease. Around 1 out of every 5 people who gets COVID-19 becomes seriously ill and develops difficulty breathing (WHO, 2020).

Observations, case studies and some case series suggest COVID-19 can affect the feet or lower limb (Mazzotta and Troccoli, 2020; Fernandez-Nieto *et* al., 2020; Landa *et* al., 2020; Kolivras *et* al., 2020). The lesions are described as Chilblains-like lesions, also known as perniosis (Landa *et al., 2020).* In a series testing of 63 patients, six testing were positive for COVID-19. The series showed that feet alone were affected most frequently (85.7%), followed by feet and hands (7%) together (Piccolo *et al.*, 2020). Skin manifestations have been the focus of these reports, which have been described as presenting as rashes: petechial, erythematous, chilblains, and ischemic. In a study, data was collected from 88 hospitalized COVID-19 positive patients in Italy. Out of these, in 18 patients Cutaneous manifestations occurred, with the trunk cited as the primary involved area (Recalcati, 2020). In a study from Wuhan, China, seven critical COVID-19 adult patients showed limb ischemia and gangrene in the feet and hands, which accounted for 21% of critically ill patients hospitalized at the same time (Zhang *et* al., 2020). It will take some time to fully understand the exact mortality rate of COVID-19. The crude mortality ratio (the number of reported deaths divided by the reported cases) calculated by the data we have so far is between 3-4%, the infection mortality rate (the number of reported deaths divided by the number of infections) will be lower (WHO, 2020).

13 Complications

The pandemic, COVID-19 appears to be more severe in patients with underlying disease conditions like hypertension, cardiovascular disease, and diabetes (Guan *et al.*, 2020) which means complications are more common in patients with comorbidities. Complications witnessed included acute lung injury, ARDS, shock, acute kidney injury, multiple organ failure and death (Hui *et al.,* 2020).

14 Pathology

Depending upon the severity of disease four types of viral pneumonia can be observed. In minor pneumonia, minor serous exudation and minor fibrin exudation is seen. In case of mild pneumonia, pulmonary edema, large atypical pneumocytes, pneumocyte hyperplasia interstitial inflammation with lymphocytic infiltration and multinucleated giant cell formation is major finding. While diffuse alveolar damage (DAD) with diffuse alveolar exudates which is the cause of acute respiratory distress syndrome (ARDS) and severe hypoxemia is seen in severe pneumonia. While another type of pneumonia called healing pneumonia is also seen in which organization of exudates in alveolar cavities, plasmocytosis in BAL and pulmonary interstitial fibrosis are major findings (Giani *et al.*, 2020). There is disseminated intravascular coagulation (DIC), thromboembolism and leukoerythroblastic reaction (Henry *et al.*, 2020; Mitra, *et al.*, 2020; Lillicrap *et al.*, 2020).

15 Diagnosis

Diagnosis is done with the help of history, clinical signs and serological testing. A person with fever, sore throat and cough and having a history of travel to China or contact with patients with similar travel history or other areas of persistent local transmission is called a 'suspect case'. However, cases may be asymptomatic or even without fever. A confirmed case is a suspect case with a positive molecular test is real-time reverse transcription polymerase chain reaction (rRT-PCR) is considered the standard method of testing. Diagnostic kits based on PCR are used in serodiagnosis of COVID-19 (Jin *et al.*, 2020).

Other laboratory investigations are usually non-specific. Other laboratory investigations are usually non specific. The chest X-ray (CXR) usually shows bilateral infiltrates but may be normal in early disease. The CT is more sensitive and specific than X-ray. The CT imaging generally shows infiltrates, ground glass opacities and sub segmental consolidation (Huang *et al.*, 2020, Jin *et al.,* 2020). It is also abnormal in asymptomatic patients/ patients with no clinical evidence of lower respiratory tract involvement. In fact, in suspect cases with negative molecular diagnosis, abnormal CT scans have been used to diagnose COVID-19 (Huang *et al.*, 2020). There is usually lymphopenia with lymphocytic count < 1000 in patients of COVID-19. The ESR and CRP are usually elevated while pro-calcitonin level is normal. Platelet count is usually normal or mildly low. The ALT/AST level, D-dimer level, creatinine level, CPK level, LDH level and pro-thrombin time elevation also has been reported (Henry *et al.*, 2020). Moreover, it is also reported that elevated values of lactate dehydrogenase (LDH) and bilirubin is frequently associated with decreased hemoglobin concentration in patients with severe and fatal COVID-19 (Henry *et* al., 2020). Moreover, ICU patients showed higher plasma levels of IL-2, IL-7, IL-10, IP10, GSCF, MCP1, MIP1A, and TNF-α than non-ICU patients. Also, initial plasma IL-1β, IL-1Rα, IL-7, IL-8, IL-9, IL-10, basic FGF, GCSF, GMCSF, IFNγ, IP10, MCP1, MIP1A, MIP1B, PDGF, TNF-α, and VEGF were found to be higher in 2019-nCoV-infected patients as compared to healthy controls (Huang *et al.*, 2020).

16 Differential Diagnosis

The most common symptoms being reported are fever, dry cough or chest tightness and dyspnoea. Thus, they are indistinguishable from other respiratory infections. Hence the differential diagnosis of COVID-19 should be done with all types of respiratory viral infections like influenza, parainfluenza, respiratory syncytial virus (RSV), adenovirus, human metapneumovirus, atypical organisms (mycoplasma, chlamydia) and bacterial infections. Through routine lab tests, it is not possible to differentiate COVID-19 from these infections clinically. Therefore, here travel history i.e., a history of travel to China or contact with patients with similar travel history or other areas of persistent local

transmission becomes important (Jin *et* al., 2020). Then, the serodiagnosis via PCR is confirmatory (Jin *et al.*, 2020)

17 Treatment

Since, it is a viral disease Treatment is essentially supportive and symptomatic. There are no medicines that have been shown to prevent or cure the disease while some traditional, western or home remedies may provide comfort and alleviate symptoms of mild COVID-19. Self-medication with any medicines, including antibiotics, as a prevention or cure for COVID-19 has not been recommended by WHO. However, both western and traditional medicines are undergoing clinical trials. For development of medicines and vaccines to prevent and treat COVID-19 WHO is coordinating efforts (WHO, 2020). Antibiotics do not work against viruses as they are effective only on bacterial infections. Therefore, Antibiotics should not be used as a means of prevention or treatment of COVID-19. To prevent or treat secondary bacterial infections which can be a complication of COVID-19 in severely ill patients, physicians will sometimes prescribe antibiotics. They should only be used on prescription of a physician to treat a bacterial infection (WHO, 2020). Antibiotics and antifungal are required only if co-infections are suspected or proven. The role of corticosteroids is yet unproven while WHO and current international consensus opposes their use. However, in COVID-19 ARDS, Chinese guidelines do recommend short term therapy with low-to-moderate dose corticosteroids (Russell *et al.*, 2020; Zhao *et al.*, 2020). In the Chinese guidelines, recommendations about using traditional Chinese herbs also find place (Zhao *et al.*, 2020). In hypoxic patients, provision of oxygen through nasal prongs, face mask, non-invasive ventilation or high flow nasal cannula (HFNC) is indicated. Mechanical ventilation and even extra corporeal membrane oxygen support may be needed. In patients with complication of acute renal injury, renal replacement therapy may be needed. Several drugs including lopinavir-ritonavir, remdesivir, hydroxychloroquine, and azithromycin have been tested in clinical trials (Cao *et. al.*, 2020; Gautret *et. al.*, 2020; Zhou *et. al.*, 2020) but none of them have been proven to be a definite therapy.

18 Prevention and control

Using proper preventive measures is the only effective way to break the chain of transmission of virus and to reduce the chances of getting infection. These includes staying at home, washing hands with soap and water often and for at least 20 seconds (Soap helps in inactivating the virus by destabilizing the lipid bilayer of virus) and if soap is not available apply an alcohol-based hand sanitizer with at least 60% alcohol, keeping distance from others, avoiding crowded places, practicing good respiratory hygiene, and avoiding touching the nose, eyes or mouth with unwashed hands. Along with following hygienic measures strictly one should boost up its natural immunity to lessen the chances of getting infection. The best way to protect others and yourself is practicing hand and respiratory hygiene is very important at all times. One should follow social distancing. If you have been in close contact with a person with COVID-19, you may get infection. Close contact means that you have been in a distance of less than 1 metre from those who have the disease. In these cases, it is best to stay at home. However, if you live in an area in which malaria or dengue fever is prevalent then it is important that you should not ignore symptoms of fever. Seek for medical help as soon as possible. And when you attend the health facility, if possible, wear a mask and keep at least 1 meter distance from other people and avoid touching surfaces with your hands. Help the child stick to this advice if it is a child who is sick. But if you do not live in malaria or dengue fever prevalent area, then if you feel sick, even with very mild symptoms like fever and headache, you must self-isolate yourself. And in condition in which you don't even think that you have been exposed to COVID-19 but develop symptoms, then self-isolate and monitor yourself because in the early stages of

the disease when you just have mild symptoms you are more likely to infect others, therefore you must go for early self-isolation. If you do not have symptoms but previously you have been exposed to an infected person then self-quarantine for 14 days. In COVID-19 confirmed case and even after symptoms have disappeared; isolate yourself for 14 days as a precautionary measure because it is not yet known exactly that after recovery from the disease, for how long people remain infectious (WHO, 2020). On self-isolation, follow national advice. One should use mask only if a person is coughing or sneezing or when one is taking care of someone with a suspected infection (WHO, 2020). Several countries like China, Spain, have recommended that healthy individuals wear face masks or cloth face coverings at least in certain public settings.

19 Conclusion

The COVID-19 is a fast spreading pandemic disease of major public health concern. The virus is mutating with time as well as the clinical presentation of disease is also changing with new upcoming cases. The virus was initially thought to involve only respiratory system but gradually with subsequent researches it is found that this disease not only involves respiratory system but also involve vascular system, nervous system, digestive system as well as cutaneous system. Its clinical signs vary, ranging from asymptomatic state to acute respiratory distress syndrome and multi organ dysfunction. Since, it's a viral disease so only symptomatic treatment and supportive therapy is the option. Till now, no vaccine has been developed against COVID-19. So, prevention is the only way to protect ourselves. One should strictly follow all the preventive measures as directed by WHO and CDC and along with this, one should boost up its natural immunity to lessen the chances of getting infection, then only we shall be able to overcome this situation.

20 Competing Interests

The authors declared that no conflict of interest exist in the publication.

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