PATHOPHYSIOLOGY AND DIAGNOSTIC APPROACH OF COVID-19 - AN OVERVIEW

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Abstract

Coronavirus has become one of the major concern worldwide after its sudden outbreak in Wuhan (China); which mainly targets human respiratory system. It's been identified as zoonotic pathogen based on its similarities with SARS, hence termed severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2); which further, provisionally named as 2019-nCoV or Novel coronavirus. Since, its emergence at the end of 2019, it promptly spreading across China, then other countries causing a world-wide outbreak and became the major concern for public health. In the past two decades, the emergence of Novel coronavirus (COVID-19) been marked as 3rd pathogenic zoonotic-virus in human population after SARS-CoV and MERS-CoV. With an unusual and sudden outbreak in Wuhan (China), Coronavirus disease (COVID-19) is a pandemic disease as affirmed by World Health Organization (WHO) on 11 March, 2020. In this review, we will discuss and compare the structural features of SARS-CoV, MERS-CoV and COVID-19 pathogenesis and its detection.
Keywords: *corona virus, COVID-19, SARS, MERS, SARS-CoV, Pneumonia*

Introduction

In December 2019, about 41 cases of pneumonia with indefinite etiology been admitted to hospitals in Wuhan city, China (Lu *et al*., 2020). In those patients, most notable clinical symptoms include high-grade fever followed by dry cough, dyspnea and bilateral lungs infiltrates on image screening. All cases were from Wuhan's Huanan Seafood Market, popular for fish, bats, poultry, snakes trading1. The virus was isolated from the patients' throat swab samples and were morphological identified as genus beta-coronavirus, when observed under electron microscope and finally named, novel coronavirus (2019-nCoV) (Xu *et al*., 2020). The World Health Organization (WHO) named this pandemic disease as COVID-19 and declared it as global emergency, on 30th January, 2020 (Sohrabi *et al*., 2020). COVID-19 is not first coronavirus outbreak of beta-coronaviruses, in the past two decades, two other outbreaks of different CoV's have emerged named, Severe Acute Respiratory Syndrome Coronaviruses (SARS-CoV) and Middle East Respiratory Syndrome (MERS-CoV) (de Wit *et al*., 2016). In 2002-2003, SARS-CoV infected about 8,000 individuals with a fatality rate of about ~10%, whereas MERS, with a fatality rate of ~36% have infected more than 1,700 individuals (de Groot *et al*., 2013; Zaki *et al*., 2012). At present, novel coronavirus has spread worldwide and considered to be less fatal than other coronaviruses; including both SARS and MERS. Overall, research has suggested that 2% of the population are carriers of CoV and these viruses are susceptible to infect 5%-10% of acute respiratory diseases. Apart from SARS-CoV and MERS-CoV, novel coronavirus (COVID-19) is the seventh member of coronavirus family, proficient in infecting humans. This review will focus on understanding the structural features and virology of MERS, SARS, and SARS-CoV-2 and will compare their fatality rate to provide a reference for its preventive measures to cope up from pandemic disease.
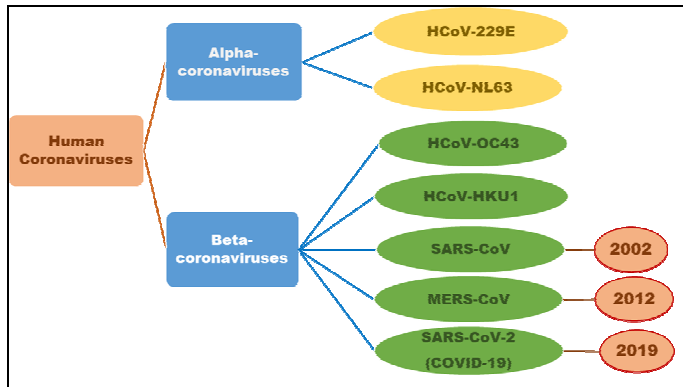
Coronavirus

Coronavirus, an enveloped RNA virus, are broadly distributed among the human population and other mammals responsible for causing respiratory, hepatic and neurologic diseases (Weiss and Leibowitz, 2011; Schildgen, 2018). They are considered as human pathogens since 1960s, but this virus's family gained infamy in year 2002-2003 with its first outbreak in the form of SARS epidemic in Guandong Province (China) (Zhong *et al*., 2003), followed by emergence of 2012 MERS-coronavirus in the Middle East9. In the recent outbreak of China, it again gained tremendous attention by creating a pandemic and epidemic emergence around the globe in the form of novel coronavirus1-2. Early studies of emergence have reported, the transmission of infection is initially through animal-to-human; hence, were of zoonotic origin; followed by human-to-human transmission through direct contact or droplets (Wang *et al*., 2020).

Belonging to Coronaviridae family, coronaviruses are single-stranded, non-segmented, positive-sense RNA-genomes (+ssRNA), comprising of about 26-32 kilobases, thus representing the longest identified viral RNA genome and probably, one of the most stable RNA (Masters and Perlman, 2013). On the basis of electron-microscopy images, they appeared as spherical virions, posing a core shell and crown-like surface projections similar to crown-like strucuture, or corona (in Latin); hence named coronaviruses (Schildgen, 2018; Masters and Perlman, 2013). Precisely, four genera of coronaviruses had been identified as α,β,γ,δ-CoV as alpha, beta, gamma, and delta-coronaviruses, respectively. The α-CoV and β- CoV are identified as human- susceptive virus (HCoV) while γ- CoV and δ- CoV are birds or rodents infecting viruses (Guo *et al*., 2020). Currently, different strains of CoV infecting humans have been identified in which, HCoV-229E and HCoV-NL63 comes under genera of α-CoV while HCoV-OC43, HCo-HKU1, MERS-CoV, and SARS-CoV belongs to β- CoV genera (Fig.1). Under the alpha- and beta- CoV genera, HCoV-229, HCoV-NL63, HCoV-OC43, and HCoV-HKU1 prototypes are known to cause mild-respiratory asymptomatic infections due to its low pathogenicity, while β-CoV prototypes- SARS and MERS lead to potentially fatal and severe respiratory-tract infections (Yin and Wunderink, 2018). On basis of current sequence databases, all HCoV originates from animals i.e. bats, rodents (Usman 2018).

Currently, few of the species from alpha- and beta-corona viruses were identified only in bats, hence proving them to be major natural reservoirs (Woo *et al.*, 2012; Vyas *et al.*, 2017). As identified from the previous researches, SERS, MERS and COVID-19 belongs to the genera of betacoronaviruses. Within the genera of β- CoV's, four sub-genera or lineages are recognized as A, B, C, and D viruses. COVID-19, belongs to B-lineage of the beta-coronaviruses and are closely related to SARS-CoV.

Fig. 1 : Genera of Human Coronaviruses (Year of their outbreak)

Structural Morphology of Coronaviruses

All coronaviruses resembles in the organization and genome expression i.e. genome contains variable number of open reading frames i.e. 6-11 ORF's. The 5'terminus (first ORF1a/b), about 2/3rd of viral genome, encodes for two large polyproteins, pp1a/pp1ab (1a/1ab). These polyproteins are, further, translated into 16 non-structural proteins (nsp 1-16), forming a replication-transcription complex (RTC) in the double membrane vesciles (Snijder *et al.*, 2006). While the other ORF's, located at 3'-terminus, are translated into four major structural proteins, such as, envelope (E), spike glycoprotein (S), membrane (M), and nucleocapsid (N), arranged in a manner – 5'-1a/1ab-S-E-M-N-3'. Besides these structural proteins, different CoV genera encodes for accessory and other structural proteins-HE (Haematoggglutinin esterase) protein, 3a/b protein & 4a/b proteins (Chen *et al.*, 2020). The nucleocapsid protein complexes form a helical capside like structure with genomic RNA, identified within the viral envelope. While, the trimers of glycosylated or spike proteins form protrusions (peplomers) providing the Virions its crown like morphology. Although, these spike proteins plays a crucial role in binding to the hosts' receptor cells and regulates the host tropism (Li, 2016). Thus, spike proteins prove to be a significant determinant for understanding the pathogenesis of infection.

Comparing the genomic sequences of coronaviruses

The genome sequences isolated from COVID-19 patient, worker from seafood market from Wuhan, shows its size of about 29.29kb, while that of SARS-CoV and MERS-CoV were 27.9 kb & 30.1 kb, respectively (Wu *et al.*, 2020). Previous studies have suggested that spike proteins bind differently on host' receptors through receptor-binding domains (RBD's) leads to the viral attachment. SARS-CoV uses angiotension-converting enzyme-2 (ACE-2) receptor of host's cells and CD209L (L-sign) as an alternative for binding (Ge *et al.*, 2013); whereas MERS-CoV make use of dipeptidyl peptidase-4 (CD-26) receptor for causing infection on hosts' cell (Zhou *et al.*, 2020). Analysis performed on the

nucleotide sequences suggested that few 2019-nCoV genes shares less than 80% sequence identity with SARS-CoV. Though, the amino acid sequences of the replicase domains in ORF1ab were about 94.4% identical between SARS-CoV and 2019-nCoV, proposing that these two viruses belong to the same genera (Velavan *et al.*, 2020). Also, 2019-nCoV is 96% identical with SARS- like bat-CoV when studied at the whole genomic level (Velavan *et al.*, 2020).

On comparing these three strains of beta-CoV, the genomes of all founds to be identical, with only difference of five nucleotides in 2019-nCoV. This genome is annotated to possess 14 ORF's that encodes for 27 proteins. The ORF1ab and ORF1a at 5'-terminal, encoding pp1ab/pp1a, comprised of 15 nsps forming nsp1-nsp10 and nsp12-nsp16 in COVID-19, in contrary to other beta-CoV. Along with nsps, the 3'-terminus contains four basic structural proteins (S, E, M, and N) and eight accessory proteins (3a, 3b, p6, 7a, 7b, 8b, 9b, and orf14). Since, 2019-nCoV belongs to beta-CoV, its amino acid levels are identical to that of SARS-CoV, but with few notable differences. For instance, 8a accessory protein, present in SARS-CoV while absent in 2019-nCoV. In SARS-CoV, 8b protein comprise of 84 amino acids while present with 121 amino acids in 2019-nCoV; 3b protein contains bigger sequence in SARS-CoV i.e. 154 amino acids as compared to 2019-nCoV. The genomic analyses suggest that SARS-CoV-2 evolved from a strain found in bats, although, its origin is still not understood. The potential amplifying mammalian host, intermediate between bats and humans, is, however, unknown. Since the mutation in the original strain could have directly triggered virulence towards humans, it is not certain that this intermediary exists (Cascella *et al.*, 2020).

Pathogenicity

Coronaviruses (CoVs) possess an enveloped, single, positive-stranded RNA genome which encodes for four membrane proteins, namely spike (S), envelope (E), membrane (M) and nucleocapsid (N) proteins 3-5 (Fehr and Perlman, 2015). With regard to pathogenicity, S proteins are essential for viral entry into host cells (Du *et al.*, 2017). SARS-CoV binds to the angiotensin-converting enzyme (ACE)2 which is present on onon-immune cells, such as respiratory and intestinal epithelial cells, endothelial cells, kidney cells (renal tubules) and cerebral neurons and immune cells, such as alveolar monocytes/macrophages (Kuba *et al.*, 2005). Of note, CD209L or liver/lymph node special intercellular adhesion molecule-3-grabbing non-integrin (SIGN) and dendritic cell (DC)-SIGN are alternative receptors for SARS-CoV but with lower affinity (Jeffers *et al.*, 2004). In the case of MERS-CoV, S proteins bind to the host cell receptor dipeptidyl peptidase 4 (DPP4 or CD26) which is broadly expressed on intestinal, alveolar, renal, hepatic and prostate cells as well as on activated leukocytes38. Then, viruses replicate in target cells with release of mature virions, which, in turn, invade new target cells (Qinfen *et al.*, 2004). Evidence has been provided that SARS-CoV proteins are cleaved into two subunits, S1 and S2, respectively, and the amino acids 318-510 of the S1 represent the receptor-binding domain (RBD) which binds to ACE2 (Qu *et al.*, 2005). Quite importantly, in the context of RBD there is the receptor-binding motif (RBM) (amino acids 424-494), which accounts for complete binding to ACE2 (Li *et al.*, 2005). Moreover, by means of two residues at positions 479 and 487 RBD allows virus progression and

tropism40, 41. In the case of MERS-CoV, its RBM binds to DPP4 with residues 484-567, thus, suggesting that its RBD differs from that of SARS-CoV (Li *et al.*, 2005). In a very recent paper, Wan and associates have investigated the receptor recognition by COVID-19 (a new term to indicate the 2019-nCoV in Wuhan) on the bases of structural studies (Wan *et al.*, 2020). In this respect, the sequence of COVID-19 RBM is similar to that of SARS-CoV, thus, implicating that ACE2 may represent the binding receptors for COVID-19. Furthermore, gln493 residue of COVID-19 RBM seems to allow interaction with human ACE2, thus, suggesting the ability of this virus to infect human cells. According, to Wan and associates structural analysis (Wan *et al.*, 2020), COVID-19 binds to human ACE2 with a lesser efficiency than human SARS-CoV (2002) but with higher affinity than human SARS-CoV (2003). Furthermore, same authors predicted that a single mutation at the 501 position may enhance the COVID-19 RBD binding capacity to human ACE2 and this evolution should be monitored in infected patients (Wan *et al.*, 2020). These predictive findings by Wan and associates44 are confirmed by two contemporary studies by Letko and Muster and Peng and associates. In particular, the report by Peng and associates, points out the possible origin of COVID-19 from bats (Letko and Munster, 2020).

From a pathogenic point of view, evidence has been provided that binding of S2 to ACE2 receptor leads to its down-regulation with subsequent lung damage in the course of SARS-CoV infection (Kuba *et al.*, 2005). Down-regulation of ACE2 causes excessive production of angiotensin (ANG) II by the related enzyme ACE with stimulation of ANG type 1a receptor (AT1R) and enhanced lung vascular permeability (Imai, *et al.*, 2005). In particular, same authors have reported that recombinant ACE2 could attenuate severe acute lung injury in mice.

In the previous paragraphs, the presence of ACE2 on immune cells has been pointed out and, by analogy to epithelial cells, this receptor may also be down-regulated following viral entry. Therefore, in CoV-infected animal models and in infected humans further investigations are required to clarify a possible reduced expression of ACE2 on immune cells. In fact, in the course of SARS-CoV infection, a number of immune disorders have been detected. Three reports have demonstrated the ability of CoV to inhibit interferon (IFN)-α production in the course of SARS acting as IFN antagonist (Lu *et al.*, 2011). In senescent Balb/c mice, depletion of T lymphocytes is associated to more severe interstitial pneumonitis and delayed clearance of SARS-CoV, thus, suggesting a protective role played by these cells (Chen *et al.*, 2010). In this connection, both SARS-CoV and MERS-CoV have been shown to induce T cell apoptosis, thus, aggravating the clinical course of disease (Yang *et al.*, 2005). Quite interestingly, memory CD8+ T cells specific for SARS-CoV M and N proteins have been detected up to 11 years post-infection (Ng *et al.*, 2016). As far as humoral immune responsiveness is concerned, evidence has been provided that S1 subunit from MERS-CoV is highly immunogenic in mice (Ababnes *et al.*, 1019). Moreover, monoclonal antibodies have been shown to be highly neutralizing against MERS-CoV replication and endowed with post exposure effectiveness in susceptible mice (Chen *et al.*, 2017). Human neutralizing antibodies have also been isolated from a recovered patient, thus, suggesting the role of humoral immunity in the control of the persistence of CoV in the host (Niu *et al.*, 2018). In particular, IgG response occurs early in infection and its prolonged production may serve for virus clearance during recovery also in view of the absence of viremia in convalescent sera from SARS patients60.

There is evidence that ACE2 protects from severe acute lung failure and operates as a negative regulator of the renin-angiotensin system (RAS) (Imai *et al.*, 2005; Li *et al.*, 2020; Oudit *et al.*, 2003). It is well known that ANG II via activation of the AT1R promotes detrimental effects on the host, such as, vasoconstriction, reactive oxygen species generation, and inflammation and matrix remodeling. ACE2 counterbalances the noxious effects exhibited by ANG II and AT1R via activation of AT2R which arrests cell growth, inflammation and fibrosis (Tiao *et al.*, 2018).

Taken together, these evidences suggest that CoV-induced down-regulation of ACE2 activates RAS with collateral damage to organs, such as lungs, in the course of SARS-related pneumonia. Then, putative therapeutic measures aimed at increasing ACE2 levels on respiratory epithelial cells should be taken into serious consideration. Quite interestingly, over the past few years, three key papers have demonstrated the ability of a polyphenol, resveratrol (RES), to experimentally deactivate the RAS system in maternal and post-weaning high fat diet, arterial ageing and high fat diet, respectively (Tiao *et al.*, 2018; Kim *et al.*, 2018). In all these experimental models, RES led to an increase of ACE2 with reduction of organ damage, such as liver steatosis and aorta media thickness and decrease of adipose tissue mass, respectively (Mehta *et al*, 2019). As far as the mechanism of action of RES is concerned, this polyphenol is able to activate sirtuin (Sirt)1 (Zordoky *et al.*, 2015; Baur *et al.*, 2006). In turn, Sirt1 down-regulates AT1R expression via ACE2 up-regulation (Kim *et al.*, 2018; Miyazaki *et al.*, 2008). Of importance, Lin and associates (Lin *et al.*, 2017) have demonstrated the ability of RES to in vitro inhibit MERS-CoV infection of Vero E6 cells, thus, prolonging cell survival in virtue of an anti-apoptotic mechanism. These findings suggest a direct antiviral effect exerted by RES. It would be very interestingly to evaluate the direct effects of RES on COVID-19, in vitro.

The bulk of data above discussed strongly suggest, that RES, as an activators of ACE2, should be investigated in animal models of CoV-induced severe pneumonia, also taking into account the anti-oxidant, anti-inflammatory and immunomodulating effects exerted by polyphenols (Magrone *et al.* 2020). Then, successful animal studies may pave the way for RES-based human trials in COVID-infected patients.

Diagnosis

The diagnosis of any pathological condition is most important to start its management. In the case of COVID-19, serology based diagnostic tests are not very helpful during peak of the infection. The serological tests can be performed to evaluate the immunoglobulin titer against SARS-CoV2, especially IgG (Letko and Munster, 2020).. The presence of SARS-CoV-2 in the host body can be detected by nucleic acid-based reverse transcriptase polymerase chain (RT-PCR) reaction. There are some other improved methods also exist for the detection of SARS-CoV-2 such as microarray-based techniques, Real time-quantitative PCR, CRISPR-Cas13 based tools. In the current epidemic time, the detection and identification of COVID-19 RNA genome is one of the useful tools in the diagnosis, which is very helpful for the

management of infection source as well as to help patients for better recovery from the illness (Katoch *et al.*, 2020). Hence the need of accurate and fast detection of coronavirus is need of today's world. Along with the growth of molecular biology and genetic engineering techniques, the nucleic acid-based identification and detection methodologies also grows rapidly and todays it becomes a revolution especially for virus detection. Among all the nucleic acid-based methods, the polymerase chain reaction (PCR) based methodologies are very much accurate, having high specificity and sensitivity and very fast. Dues to these reasons the PCR is a monetary standard method for virus detection. Along with PCR based methods a number of non-PCR based methodologies also exist such as loop mediated isothermal amplification (LAMP) and nucleic acid sequence-based amplification. These are isothermal nucleic acid amplifications methods developed for the spotting and identification of COVID-19 RNA genetic material. Here we reviewed a number of coronavirus detection methodologies and approached and hope this will helps scientific community for better and fast and accurate detection of viral RNA. The Some biochemical parameter also gives semiquantitive method for the COVID-19 like c-reactive proteins (CRP). Currently we also use a rapid COVID-19 detection kit which detects the present of IgG or IgM in the host body against SARS-CoV-2 N-Protein. There are a number of limitations is associated with this method and hence there is a need of fast more sensitive and specific diagnostic tool is needed which can detect a greater number of samples in less time span. Although the RT-qPCR is a sensitive and specific tool for the detection of coronavirus but its false positive rate cold not be ignored. Hence, a number of clinicians has suggested that CT scanning can be one of the compulsory auxiliary diagnostic tools due to its sensitivity. Combination of repetition of RT-qPCR along with the chest CT scanning should be performed in the patients with symptoms of SARS-CoV2 but negative RT-qPCR. The high-resolution CT scanning of chest (HRCT) is necessary for the preliminary diagnosis of the severity of COVID-19 in the patients infected with SARS-CoV2. A common visualization of CT scanning shows the bilateral pulmonary parenchymal ground-glass and consolidative pulmonary opacities, sometimes with a rounded morphology and a peripheral lung distribution (Khatik *et al.*, 2018. Lung also got affected in the patients with SARS-CoV and MERS-CoV infections and the CT scanning of chest reveals that the abnormalities progresses along with the ground-glass opacities and consolidation. A similar type of results is also seen in the case of SARS-CoV-2 infections. These findings suggest that the chest CT scanning can be a great helpful in the diagnosis of COVID-19 in the area with high prevalence. One of the major abnormalities of CT scanning is the it cannot differentiate between the pneumonia caused due to other viruses and hysteresis of abnormal CT imaging."

Conclusion

In Current scenario the outbreak COVID-19 has been declared as Health emergency worldwide. The number of cases COVID-19 is continuously going up day by day . The total global number of COVID-19 cases has surpassed 1,278528 and the deaths due to COVID-19 worldwide is 69,757. The presence of SARS-CoV-2 in the host body can be detected by nucleic acid-based reverse transcriptase polymerase chain (RT-PCR) reaction. There are some other improved methods also exist for the detection of SARS-CoV-2 such as microarray-based techniques, Real time-quantitative PCR, CRISPR-Cas13 based tools. Some biochemical parameter also gives semiquantitive method for the COVID-19 like c-reactive proteins (CRP). Currently we also use a rapid COVID-19 detection kit which detects the present of IgG or IgM in the host body against SARS-CoV-2 N-Protein. There are a number of limitations is associated with this method and hence there is a need of fast more sensitive and specific diagnostic tool is needed which can detect a greater number of samples in less time span. Currently there is no vaccine available and the main treatment for tackling this pandemic is supportive. Although quarantine alone won't suffice to prevent the spread of COVID-19, and the influence of this viral infection is one of increasing concern all over the globe (Wan *et al.*, 2020). After the outbreak of epidemics SARS and MERS, significant research came into existence to develop a new antiviral agents and vaccines that can target CoVs proteases, polymerases, MTases, as well as the proteins that facilitated entry inside host, but none passed the clinical trials. However blood of recovering patient and obtaining Plasma and antibodies from it can be used in treatment of COVID‑19. Currently COVID‑19 has spread as a worldwide pandemic with no vaccine available till date.

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