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Perspective

Covid-19 Outbreak on The Rise - Anticipating Treatment Strategy

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Abstract

The outbreak of novel coronavirus from Wuhan, China has become the global concern as more than 2000 people has been died and nearly 75,000 has been proved infected. History showed that, such outbreak is continually happening in every decade or two. Hence, the advancement in science and technology should be prepared of such situation. On the other hand, past studies suggested that the coronavirus outbreak mainly occurred or transmitted from animals and it very crucial to avoid consumption or use of such animals in normal life. The alertness should be strict because the virus is able to change their genetic makeup when it transmits from one animal to the other and, hence the protein target for antiviral agent also need a change. At this situation, it is necessary to find out as much target as possible to use the existing drugs or antiviral agent to control the spread.

Keywords: Coronavirus; Covid-19

The name "coronavirus," devised in 1968, is derived from the "corona"-like or crown-like morphology observed for these viruses in the electron microscope [1]. Time and again, this virus has created chaos in human and animal health. Numbers of coronaviruses have been identified and reported in the database, most of which can circulate among animals, including pigs, camels, bats, and cats. In some cases, the virus can jump to humans and can cause disease, termed as a spillover event. Until now, seven different types of coronaviruses have been found to cause human disease [2]. Among them, four viruses viz. 229E, OC43, NL63, and HKU1 were found to cause mild symptoms, while three of the coronaviruses have shown serious outcomes in people. Those three coronavirus are SARS (severe acute respiratory syndrome) (emerged in late 2002 and disappeared by 2004), MERS (Middle East respiratory syndrome) (emerged in 2012 and remains in circulation in camels) [2] and the COVID-19 which has recently emerged in December 2019 from Wuhan city, China and now has become a global concern and the world is struggling to contain its spread [3]. As the number

of cases of novel coronavirus has heightened with nearly 75000 and more than 2000 deaths, the World Health Organization (WHO) has already declared an emergency.

Wuhan coronavirus is a novel coronavirus which is a member of the coronavirus family that has never been encountered before. Similar to other coronaviruses, it has also found to be originated from animals showing ~89% similarity with Bat SARS-like coronavirus isolate Bat-SL-CoVZC45 in Chinese bats (https://blast.ncbi.nlm.nih.gov/Blast.cgi). It is also noted that most of the early diagnosed patients either worked or frequently shopped in the Huanan seafood wholesale market in the center of the Chinese city, which also sold live and newly slaughtered animals, including bats and snakes. Scientists suggest that the origin of such deadly viruses (such as SARS, MERS, COVID-19) are usually by animal host most probably bats. However, a recent study also predicts it could be a snake and possibly transmitted via intermediary species such as pigs, camels, or cats [4]. The Wuhan Coronavirus COVID-19 has several symptoms, mainly pneumonia, cough, fever, and breathing difficulty. In a more severe case, the symptoms lead to organ failure. The antibiotic and antiviral drug does not affect it. The hospitals all over the world are providing support for lungs and other organs and supplementing fluid trying to prevent multi-organ disfunction and multi-infections. However, in this situation, the recovery of a person also depends on their immune system because it is found the most of the death was observed in those who are already in poor health conditions. The most serious aspect of this virus is due to its potential for human-to-human transmission and asymptomatic contagious. It is mentioned that Coronaviruses, like the Wuhan virus, can travel only about six feet from the infected person; however, it is not known how long they live on surfaces.

Unlike SARS, after being criticized for not sharing the information, the Chinese government is making great effort to reduce the transmission and to determine the best way to handle the deadly, rapidly spreading disease. Although there's no specific treatment for coronaviruses, and with the facts, the virus will complete its course, the doctor says, most people will recover on their own. For minor cases, the best way to treat symptoms is to take pain and fever medications, such as tylenol, aspirin or decongestant (Jay Cook, Chief Medical Officer at Providence Regional Medical Center in Washington) use a room humidifier or take a hot shower to help ease a sore throat and cough, drink plenty of liquids, and rest (CDC). China has applied major travel restrictions and quarantined the most affected cities.

The molecular function in gene ontology (GO) of coronavirus showed its involvement in transporter activity, transmembrane activity possessing ionic, carboxylic acid, organic and malate transmembrane transport activity while the biological process includes development, multicellular organismal, anatomical and tube development process (Figure1 and 2). This suggest that these viruses are involved in wide range of molecular function and biological process. However, the treatment of coronavirus has created a problem for scientists. Some of the drugs and vaccines are standing on the pipeline to be effective but successful result has not been obtained yet. Some of the promising treatment methods include vaccines, drugs that target viruses or the host cells and genetically engineered human T-cells. However, all the methods cannot be regarded as an instant solution to this global outbreak. Vaccine development is a complicated process often requiring 10 - 15 years. It needs to identify natural or synthetic antigens that could include virus-like particles, weakened viruses, or other substances derived from pathogens that can produce antibodies against those particles and stay in the bloodstream for life long. Drugs can also work effectively for viruses but it has more undesirable side effects. Viruses can enter the human cells, can replicate itself and destroy the new cells. For instance, the drugs could block virus replication inside cell; however, the virus remains dormant and could mutate or change its features to become more destructive. Another drug that induce targeted apoptosis and phagocytosis could also be effective but it takes long research that cannot provide any solution to the present situation. Another potential prevention is the development of genetically engineered human T-cells that recognize and kill infected cells and inject them into a human. However, each T-cell is specific to each person and it takes long time to develop T-cells in the laboratory. So at the present moment, two possible out ways could be considered. One is the rapid development of vaccine by combined effort globally and another is to use the existing drugs. Since it has been seen that the genomes of virus (in fact most of the microorganisms) possess some percentage of similarities. With this regard, it is possible to analyze the nucleotide sequences, amino acid sequences, some conserved sequence and its similarity with other viruses. Current report stated that anti-HIV drug is being repurposed to act against coronavirus. This can provide some clues to find some common properties of virus and use the existing drugs.

Figure 1: Gene Ontology of coronavirus family for molecular function. The sequences were blasted for Wuhan Coronavirus isolate HU-1. The sequences of coronavirus included are: NC_045512.2 (100%), MN988669.1 (100%), MN994468.1 (99.99%), MN975262.1 (99.98%), MG772933.1 (89.12%) and AY395003.1 (82.34%).

Figure 2: Gene Ontology of coronavirus family for biological process. The sequences were blasted for Wuhan Coronavirus isolate HU-1. The sequences of coronavirus included are: NC_045512.2 (100%), MN988669.1 (100%), MN994468.1 (99.99%), MN975262.1 (99.98%), MG772933.1 (89.12%) and AY395003.1 (82.34%).

Like SARS and MERS, 2019-Coronaviruses are enveloped, positive-stranded RNA viruses with approximately 30,000 nucleotides. The uniprot.org analysis showed that there are around 15000 GO in around 13750 organisms. The un-translational regions (UTR) and open reading frame (ORF) of Wuhan Human Corona Virus (WHCV) are similar to two coronaviruses; a human-origin coronavirus (SARS-CoV Tor2, AY274119) and a bat-origin coronavirus (Bat-SL-CoVZC45, 110 MG772933) with a gene order 5'-replicase ORF1ab-S-envelope(E)-membrane(M)-N-3'. It has 5' and 3' terminal sequences characteristic of the betacoronaviruses (265 nt at the 5' terminal and 229 nt at the 3' terminal region). The expected replicase ORF1ab gene of WHCV is 21,291 nt in length and contained 16 predicted non-structural proteins, followed by (at least) 13 downstream ORFs, as explained by Wu *et. al*., 2020 [5]. Further, high similarities of amino acid sequences and predicted protein structure between WHCV and SARS-CoV RBD domains suggest that WHCV may efficiently use human ACE2 as a cellular entry receptor, perhaps facilitating human-to-human transmission [6,7]. Previous study on SARS-CoV showed presence of two overlapping open reading frames (ORF1a and ORF1b), which act on translational readthrough by a-1 ribosomal frameshift mechanism. This permits the translation of the overlapping reading frames into single polyprotein pp1ab, whereas, translation without the -1 ribosomal frameshift mechanism produces pp1a cleaved by two viral proteinases,

3C-like protease (3CLP) and papain-like protease (PLP), yielding non-structural proteins essential for viral replication [8-10]. These polyproteins have important role in developing antiviral agent. As per now many antiviral drug has been tested for corona virus, however, no specificity has been successfully obtained. For example, 3CLP, PLP, RNA-dependent RNA polymerase (RdRp) and the 5′-3′ helicase [10,11], E protein (Orf4), M protein (Orf6), and N protein (Orf9) [10] has been considered as a viable target for two viruses SARS and MERS to act as viral inhibitors. Mainly replication inhibitors and entry inhibitors are commonly understood mechanism to develop antiviral agents. 3CLP papain like protease (PLP) and nsp13 (helicase) which is an essential component of the SARS-CoV replication machinery, are among the first SARS-CoV inhibitors that were discovered by screening compound libraries using a fluorogenic peptide [10,12]. The compounds such as zinc or mercury conjugates, C2-symmetric diols, peptidomimetic α,β-unsaturated esters, anilides, benzotriazole, N-phenyl-2-(2-pyrimidinylthio) acetamide, biphenyl sulfone, glutamic acid and glutamine peptides possessing a trifluoromethylketone group, pyrimidinone, and pyrazole analogues were identified to inhibit 3Cpro of picornaviruses CV-B3 (coxsackievirus), EV-71 (enterovirus) and RV- 14 (rhinovirus) (coronavirus and picornavirus dual inhibitors) [10]. Further, a yeast-based assay to screen for small molecules that block SARS-CoV replication based on their inhibition of nsp3 or PLP has also been established [13]. In that research it was observed that one compound, NSC158362, can inhibit SARS-CoV but not influenza. Likewise, inhibitors SARS-CoV helicase (termed as nsp13) are found to restrict its unwinding and ATPase activities. Among the experimented compounds, bananin, iodobananin, vanillinbananin, and eubananin were found to effectively inhibit the helicase activity of nsp13 by inhibiting the ATPase activity of the helicase with IC_{50} values in the range 0.5 - 3 μM. Recently, SSYA10-001, belonging to helicase inhibitor, was shown to exhibit a broad-spectrum activity against other coronaviruses, including MERS-CoV and mouse hepatitis virus (MHV) [10,14]. Entry inhibitors are other potential targets for the analysis of antiviral agents. One example is RFI 641, small molecules that inhibit the binding of particles at a hydrophobic pocket of the fusion (F) glycoprotein and restrict the entry of respiratory syncytial virus [15]. Similarly, Maraviroc is an entry inhibitor of HIV that targets CCR5, Enfuvirtide and SC29EK are peptides inhibit binding to the viral transmembrane protein gp41. The surface glycoprotein of SARS-CoV, SARS-S, possesses two constituents S1(receptor-binding domain (RBD)), and S2 (fusion peptide). In case of SARS-CoV, interactions of the SARS-S RBD with the cell surface receptor, angiotensin-converting enzyme 2 (ACE2) [10,16] allows the entry of virus and cleaved by cellular protease called cathepsin L has been reported [10,17,18]. However, no specific drugs have been approved for SARS or other coronaviruses. While, it is important to be noted that during SARS outbreak major drugs including ribavirin with and without corticosteroids, interferon (alfacon-1) with corticosteroids [10], and ribavirin with protease inhibitors [10,19] had shown some positive outcomes. In last several years, efforts on combinations of several medications have been performed [20] but satisfactory results have not been obtained because of high concentration dosage and adverse side effects (hemolytic anemia, elevated transaminase levels and bradycardia, depression, suicide, relapse of drug abuse/overdose) [21-23]. Numbers of companies all over the world in collaboration with WHO and other research institutes are working on developing candidates for antiviral vaccines and drugs. Among them, Novavax's MERS and Inovio Pharma's INO-4700 that bind to the major surface spike (S) protein has been developed. Similarly, Galidesivir (BCX4430) (broad-spectrum antiviral activity), Regeneron's REGN3048-3051 (bind to S-protein), several HIV protease inhibitors such as lopinavir, ritonavir and cipla have also been repurpose to use against coronavirus. Moreover, the recent information about COVID-19 virus in news have reported that the use of HIV drugs have shown good result for treatment and it is being used with positive results for lots of the infected patients in which about 21.4% (https://covid19info.live/) has been recovered in mainland China itself. Nevertheless, it could more rapidly be tested in patients infected with the new coronavirus.

Overall, past history suggest that these outbreaks are common in every decades or two. And most of the outbreaks has been found to be animal originated. It suggests that animals can be considered as the natural reservoir hosts of such viruses. Although corona virus is identified widely in bats, snakes, cats and dogs, there might be several other reservoir host of this virus which need to be studied in future. Because, COVID-19 virus was identified in Wuhan seafood and live animal market, there could other animal source of this virus. Another thing that should be considered is to ban the consumption of such wild animals and birds so as to avoid the outbreak of various diseases. Further, at this health emergency situation of COVID-19 outbreak, where thousands are being infected and hundreds are dead, the most important thing is to find the an-

tiviral agents, drugs or chemical that can work in cocktail. Since, developing vaccines, drugs and other genetic methods take longer time, it is important to use the combination of existing drug based on their target protein or cell surface receptor.

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