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COVID-19 : An Overview of Diagnostic Approach and Novel Directions for Treatment Strategies

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Abstract

Coronavirus disease (COVID-19) turned out to be one of the biggest biological disasters in the era of the 21st century in relevance to the threat faced worldwide. The pace of the spread of the pathogen is at an alarming rate and leading to the pandemic situation throughout the globe. In order to start any treatment procedure, a clear diagnosis for the identification and isolation of the disease is the utmost. Early detection and diagnosis of the pathogen are necessary for the further reduction in the outgrowth of cases among the populace. However, there are substantial advancements taking place in the technological aspects of the novel assays and the development of diagnostic applications. The clinical diagnosis meant to be the first diagnostic procedure wherein the suspect examined for the clinical manifestation such as cough, fever, difficulty in breathing, etc. and CT scan. Novel laboratory assays like real-time reverse transcriptase PCR (rRT-PCR) are fast, reliable, and sensitive tests for the detection. In cases, where there is a negative correlation with respect to the nucleic acid detection methods for the diagnosis, but the epidemiological link of the infection is effective, the tool of immunodiagnostics and serology plays a crucial role in making use of the antibodies, and viral antigens for the detection. Another approach in the identification and diagnosis of the SARS-CoV2 is the preliminary examination of the pathogen following the classical Koch's postulates and further analysis using the electron microscopy. Sequencing of the entire genome of viruses may also inform studies of the molecular epidemiology of the virus. The last part of the review article incompasses novel directions for treatment strategies.

Keywords: Coronavirus; Diagnosis; rRT-PCR; Immunodiagnostics; Serology; Microscopy

Introduction

Coronavirus (CoV) are a large subfamily of enveloped positive single-stranded RNA viruses with the largest known genome size of about 30 Kb -32 Kb, having the capacity of infecting a variety of wild and domestic animals as well as humans[1]. These are a group of viruses inside the Coronavirinae subfamily inside the family of Coronaviridae and the order Nidovirales, which are separated into three domains (1, 2, 3), initially dependent on antigenic reactivity, later affirmed by genome sequencing[2]. As of late, the International Committee on Scientific Categorization of Viruses (2009) adjusted another ordered terminology. In this context, coronaviruses are separated into four genera (alpha, beta, and gamma coronaviruses), comparing to domains 1, 2 and 3 inside the subfamily coronavirinae, while a new set of coronavirus (delta coronavirus) is suspected to be evolved from the family of birds (bats) inside the group of coronaviridae, what's more, inside the order or superfamily of Nidovirales (Figure 1)[3].

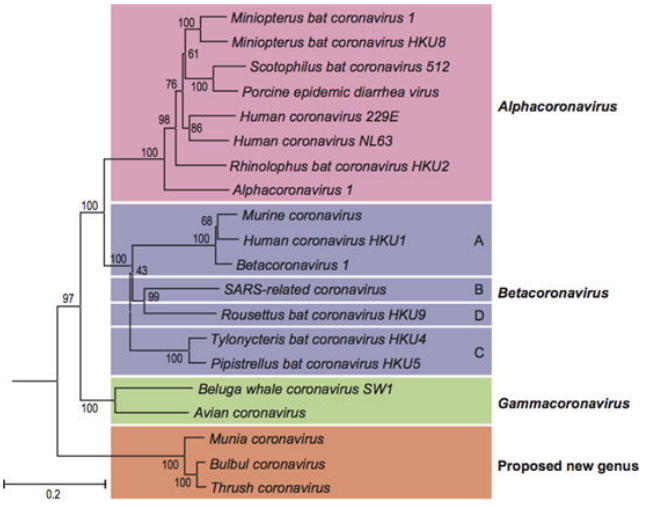

Figure 1: Phylogenetic relationships among the members of the subfamily Corona virinae.

Till date, six variants of human coronavirus have been discovered, alpha-CoVs (NL63 and 229E), beta-CoVs (OC43 and HKU1), severe acute respiratory syndrome-CoV (SARS-CoV), and Middle East respiratory syndrome-CoV (MERS-CoV) [4]. New strain of the coronavirus (SARS-CoV-2) emerges to be in prevalence and wide distribution due to the changes in their genetic diversity, increasing in

human animal interface activities and frequent recombination across the genomes [5,6]. SARS-CoV-2 is known to be the seventh virus to infect humans which are widespread and are severe disease causing organisms, while on the other hand the alpha and beta variants of coronaviruses are known to show mild symptoms [7].

Coronaviruses (CoVs) have caused a significant flare-up of human deadly pneumonia since the beginning of the 21st century. (SARS-CoV) spreading of these newly CoVs to five landmasses in 2003 and Middle-East Respiratory Syndrome Coronavirus (MERS-CoV) outbreak in 2012 in Arabian Peninsula showing that these group of viruses are zoonotic which have the capability of being transmitted from the animals to humans and from humans to humans [2,8-9]. The term 2019 novel coronavirus (2019-nCov) was referred by the World Health Organization (WHO) to emphasize the outbreak of this virus, which affected the lower respiratory tract of the patients in the city of Wuhan, China in December 2019. It was later officially named as COVID-19 and the current reference suggested it to be Severe acute respiratory syndrome coronavirus-2 (SARS-CoV 2) [5]. The SARS-CoV-2 belongs to the β genus CoVs containing at least four structural proteins: Spike (S) protein, envelope (E) protein, membrane (M) protein, and nucleocapsid (N) protein (Figure 2) [1,10]. The last part of the review article in compasses novel directions for treatment strategies to steal the scientific attention on the present approaches to combat the disease.

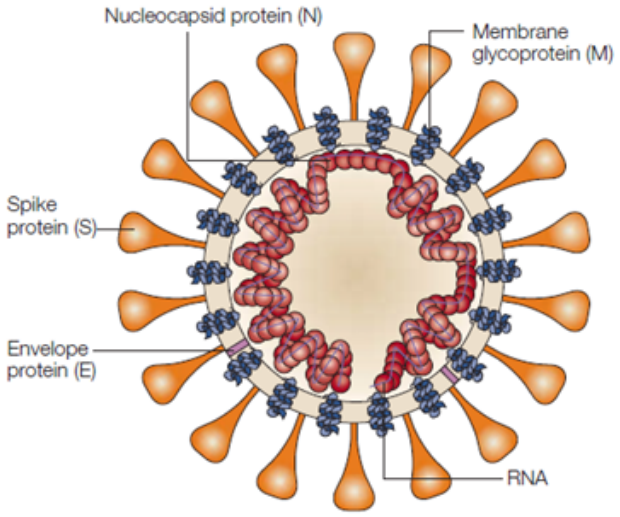

Figure 2: Ultrastructural morphology of Coronavirus. The viral proteins are enclosed in the lipid bilayer envelope inside the host cell.

Clinical manifestations and transmission

These viruses are capable of causing symptoms like fever, pneumonia, pleural infection and difficulty in breathing leading to fatality. These virus are known to affect the animals in various surroundings across the globe but the infectibility to humans was negligible [11]. Likewise, the patients with mild symptoms are known to recover sooner, while the acute cases were experienced with respiratory failure leading to death. Since there is no such drug for the treatment for the coronavirus, it is of huge impeccable threat to humans.

The pandemic ancestry of SARS-CoV is accepted to have been obtained by people from predatory wild game, for example, civet cats, which in turn are thought to have procured the infection from rhinolophid bats [12]. These viruses are spread through mammals and birds although transmission isn't by natural vectors, however – relying upon the infection species – by means of fomites or through aerogenic as well as fecal–oral courses [7]. However recent studies suggest that these viruses could be the transmitted from the bats through an intermediate host as the whole genome sequence of the bats was found to be 96% identical and 79.5% was found to be similar with SARS-CoV which clues us that bats are the most probable host of SARS-CoV2 in transmission of the disease to humans [13,14]. SARS-CoV2 spreads for the most part by air-droplets created because of coughing or sneezing of an infected individual. This can occur in two different ways: (a) Direct contact: one can get the disease by being in close contact with SARS-CoV2 patients (inside 1 Meter of the contaminated individual), particularly on the off chance that they don't cover their face when coughing or sneezing. (b) Indirect contact: the droplets reside on surfaces and garments for a long time. In this way, contacting any such tainted surface or fabric and afterward contacting one's mouth, nose or eyes can transmit the disease.

Diagnosis

The SARS-CoV2 incubation time (time between getting the disease and indicating symptoms) is 1 to 14 days. However some individuals with the disease, yet with no genuine symptoms can likewise spread the infection. The stability of the virus is detected highly stable at 4°C, but are sensitive to heat. However they were found to be stable at a variation range of pH at normal environmental conditions, but susceptible to the disinfectants [15]. However there are various ways to diagnose the disease based on the clinical diagnosis, nucleic acid based detection, immunodiagnostics, microbial examination etc [16].

Clinical diagnosis

Physical examination is the first diagnostic criteria to be taken into consideration. The most common symptoms are the fever, cough, fatigue, difficulty in breathing, viral pneumonia while the short term symptoms are diarrhea, runny nose, headache, cough and nasal congestion [3].

Physical examination

Patients are physically examined for the temperature abnormalities i.e. increase, shortness of breath, fatigue, vocal tremor. The patients with these symptoms should be isolated and quarantined. However, patients with mild symptoms may not show up positive results [13].

CT imaging

The normal CT imaging show respective pneumonic parenchyma and consolidative aspiratory opacities, now and a fringe lung dispersion. Lung association with a fringe dispersiom was found in patients with SARS-CoV and MERS-CoV diseases, and the chest CT demonstrated that infection advanced with opacities and combination, which is like that of SARS-CoV-2 contamination [17]. Concurring to those discoveries, CT checks have an extraordinary clinical analytic incentive for COVID-19, particularly in the high pervasiveness zone of SARS-CoV-2 contamination [16,18].

Nucleic acid based detection

While most of the detection portfolio makes use of the viral genetic material, this technique makes use of the molecular biology tool known as the real-time reverse transcriptase polymerase chain reaction (rRT-PCR) by amplifying specific oligo sequences from the

respiratory (nasal and throat swab samples) and the blood samples, however, the sensitivity of the test is sometimes uncut as the PCR can detect the virus if only present within the suspect's sample and hence output about the infection is unclear[12,19-20]. Another strategy in the nucleic acid detection technique, is the high-throughput sequencing, but is limited due to its high cost and maintenance.

Immunodiagnosis and serological techniques

In cases, where there is negative correlation with respect to the nucleic acid detection methods for the diagnosis, but the epidemiological link of the infection is effective, the tool of immunodiagnostics and serology play a crucial role [21]. The presence of viral proteins (antigens) in the COVID-19 infection is diagnosed by one of Rapid Diagnostic Test (RDT) from the respiratory tract of an individual, wherein the samples (serum) are used for the analysis of the immunoglobulin's (IgG and IgM) antibody detection reagents and SARS-CoV-2 antigen detection reagents using the ELISA technique [22]. However, tests to distinguish immune response reactions to COVID-19 in the populace will help the improvement of antibodies, and to add to our comprehension of the degree of disease among individuals who are not recognized through dynamic case finding and reconnaissance endeavors, the assault rate in the populace, and the contamination casualty rate [23]. Although, serological assays are not utilized for analysis of HCoV infection because of the unavailability of commercial reagents and kits, they are significant for understanding the study of disease transmission of rising HCoVs, including the conditions of asymptomatic contaminations [24].

Microbial detection

Another approach in the identification and diagnosis of the SRS-CoV2 is the preliminary examination if the pathogen following the classical Koch's postulates and further analysis using the electron microscopy[25-28]. In addition to verifying the existence of the virus, routine sampling of specimens from clinical cases can be useful for monitoring for mutations in viral genomes that could influence the efficiency in medical countermeasures, including diagnostic testing. Sequencing of the entire genome of viruses may also inform studies of the molecular epidemiology[14].

Future treatment strategies

The current scenario is that, there is no approved treatment therapy for COVID-19 is available. However, virologists and frontline clinicians have been experimenting with virus based and host-based therapeutics since the outbreaks in the China. After a plethora of research search, we herein mention few pre-existing and novel therapeutic strategies for emerging scientists to discover drugs and/or vaccines to inhibit this viral outbreak that could assist in developing novel therapeutics for COVID-19 treatment.

- One of the great signs of COVID-19 is the so-called 'cytokine storm' due to attack of SARS-Cov-2 in the lungs. A cytokine family member Interleukin-6 (IL-6) inhibitors might be valuable for patients who developed cytokine discharge syndrome. Considering, Mesenchymal Stem Cells (MSCs) therapy could contribute against SARS-CoV-2 viruses attack because of their immune modulatory, anti-inflammatory, and restorative ability linked to their stemness to the arsenal of treatments for COVID-19. For potential clinical recovery, remdesivir might be regarded early in the course of illness expeditiously before disease progression. Before concluding on efficacy, more well-designed RCTs are warranted in COVID-19 therapies.
- For optimal outcomes, antiviral therapies like remdesivir, lopinavir/ritonavir and umifenovir could be initiated before the viral replication attains its peak level. Ribavarin is generally ineffective as a monotherapy and may be beneficial an add-on therapy.
- The use of corticosteroids should be limited to indicating comorbidities. Owing to lack of data in COVID-19, IVIG is usually not recommended.
- Due to the conflicting outcomes in coronavirus studies, the efficacy of interferon is still unclear.
- Scientist should also work on investigating molecules that may target autophagy. Blocking of autophagy inhibits virus entry into cells, however, antigen presentation by macrophages blocks the activation of adaptive immunity on T cells as well as B cells. Consequently, the autophagy inhibitors would be best administered after achieving the adaptive immune response against the COVID-19, which often takes almost 5-6 days after the onset of disease.
- Chloroquine and hydroxychloroquine demonstrated in vitro inhibition of SARS-CoV-2, and whether the benefits outweigh the risk of dysrhythmias remain inconclusive.
- Apart from mediating the virus entry, ACE2 also displays protective role in the pathophysiological process of virus-induced ALI but the sequential role of ACE2 still remains unclear in the whole disease process and high-quality clinical trials and real-world data are potentially required to answer the question.
- Although, in view of its key role in disease pathogenesis and pathophysiology, ACE2 has inspired comprehensive interests and plan of action targeting ACE2 and its ligand-COVID-19 spike protein; this might render novel method in the prevention and management of COVID-19.
- Numerous epidemiologic associations evidently advocate a rational hint that the MMR vaccine may grant protection to the COVID-19 virus as well. An immediate exploration of utilizing the already available MMR vaccine in controlled studies is urgently required to demonstrate a protective benefit. Epidemiologic research indicates its efficacy equivalent as a COVID-19 vaccine, and this could be set in motion within months, probably saving thousands of lives with an earlier deployment as compared to other vaccines under development.
- Researchers are attempting cellular therapies like monoclonal antibodies (Tocilizumab, Adalimumab, Siltuximab etc.) to create antibody dependent therapies to suppress and/or neutralize SARS-CoV-2. The virus structural and genetic similarity to SARS-CoV may assist in the creation of new approaches for COVID-19 therapy.
- As discussed above, study results have envisaged that the ubiquitin-proteasome framework performs a crucial role during several stages of the coronavirus disease. N protein of SARS-CoV-2 could be debased by PA28γ in vitro. This may show that PA28γ is a controller for SARS-CoV-2 N protein debasement. This stipulates some insight for understanding the earlier debatable physiological function of the proteasome-dependent debasement of the SARS-CoV-2 N protein during pathogenesis of COVID-19. Understanding the meticulous role of PA28γ may give us new shrewdness into virus-cell interplay and lead to a more noteworthy comprehension of the pathogenicity of 2019-nCoV infection.
- Scientist should target human proteases involved in activation "Viral Spike protein". They may be transmembrane proteases, secreted proteases and intracellular Endoplasmic Reticulum (ER) proteases. Although the researches are conducting on exploration of human proteases that might activate and cleave SARS-CoV-2 Spike protein, the intracellular ER proteases can continue to activate Spike protein

and induce viral membrane fusion after initial endocytosis activated by extracellular proteases. Few drugs having ability of blocking extracellular proteases (For instance; camostat, nafamostat, and cobicistat) are now in clinical trials.

- Vaccine development in progress by Oxford University and AstraZeneca's lead candidate AZD1222 is the most advanced one among all the samples currently in development is likely to enter the third phase of the trial in the upcoming week. On the other hand, Moderna/NIAID candidate, mRNA-1273 enters the final phase of the trial study in evaluating immunogenicity, dose levels and adverse effects towards the safer efficacy, as previous trials reported safer immune response in all the volunteers tested.

Conclusion

During such crises of COVID-19 pandemic, generation of timely evidence for treatment options is crucial. Consequently, it is rational to maintain methods and public health measures until potent and effective drugs/vaccines are discovered. An appropriate immunomodulatory diet, proper mental support, adherence to standards and combinatorial therapies will be effective in the long run against COVID-19. Taking into consideration that in absence of any effective action and lack of effective therapeutic approach along with inadequate wide implementation of social distancing, the situation will remain same until 2022 with 90% of the global population affected with COVID-19 and evident mortality in over 40 million people. Moreover, future viral outbreaks resulting from pathogens of zoonotic origin are likely to continue, hence, comprehensive measures must be devised apart from curbing this outbreak. Special emphasis and endeavor to shield and decrease transmission should be employed in sensitive populations including children, health care providers, and elderly people. Owing to the weak immune system which permits rapid progression of viral infection, early mortality cases of COVID-19 outbreak were prominently seen in elderly people, Taking into account the rising incidence of CoV emergence in livestock animal populations and the identification of novel CoVs in reservoir species proves the high vulnerability of CoVs beyond public-health intervention strategies. Consequently, the design and development of vaccines for SARS-CoV-2 is equally crucial in addition to developing new drugs and clinical trials of old drugs. Experience from SARS-CoV and MERS-CoV indicates for significant emphasis on establishing animal models which can summarize various aspects of human disease and determinants of vaccine safety and efficacy.

Conflicts of Interest

Authors declare that there is no conflict of interest.

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