COVID-19: Microbiological Perspective

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ABSTRACT

Coronavirus-2019 (COVID-19), also named as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is a highly contagious disease, which has lead on to ongoing pandemic outbreak and now ended up in global public health emergency. This article will briefly review the morphology, genomic nature, and pathogenesis of this virus. In addition to it, brief description on laboratory diagnosis, approach to treatment, and preventive measures of COVID-19 based on various published research literature has been discussed.

Keywords: COVID-19, Cytokine storm, Pneumonia, Real-time PCR (RT-PCR).

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INTRODUCTION

Coronavirus-2019 (COVID-19), also named as SARS-CoV-2, which is now causing a recent outbreak of viral pneumonia, seldom ends in severe acute respiratory syndrome coronavirus. COVID-19 is the third pandemic severe respiratory disease outbreak caused by the coronavirus. In twenty-first century, there occurred two other pandemic by coronaviruses, namely the severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome coronavirus (MERS-CoV), into the human population. On 31 January 2020, the World Health Organization (WHO) officially named the disease "COVID-19." Simultaneous naming of this virus as "severe acute respiratory syndrome coronavirus 2" (SARS-CoV-2) was given the International Committee on Taxonomy of Viruses.[1]

CLASSIFICATION OF CORONAVIRUS

Coronaviruses (CoVs) are single-stranded enveloped RNA viruses. They come under order Nidovirales, family Coronaviridae, subfamily Coronavirinae, which is further subdivided into four genera, namely alpha, beta, gamma, and delta coronavirus. Beta coronavirus is further classified into five subgenera embecovirus, hibecovirus, merbecovirus, nobecovirus, and sarbecovirus. Our virus of importance, SARS-CoV-2 belongs to genus beta coronavirus and subgenus sarbecovirus. Different coronaviruses exhibit varied host range and tissue tropism. Alpha and beta coronaviruses commonly infect mammals, whereas birds and fish are more commonly infected by gamma and delta coronaviruses.

HUMAN CORONAVIRUS TYPES

Human coronaviruses 229E, NL63, OC43, and HKU1 are the ones that commonly infect people all over the world. In certain occasions, coronaviruses that infect animals can evolve and transform into a new human coronavirus. Three such examples of this zoonotic coronaviruses are SARS-CoV, MERS-CoV, and the recently emerged, 2019-nCoV. According to the WHO report, the pandemic of SARS occurred around 29 countries, which killed nearly 10% of 8,096 people affected. Compared to SARS, outbreaks of MERS are still occurring. Since 2012, MERS has caused 2,494 confirmed cases in 27 countries and killed 858 with the mortality rate of 30% people.[2,3]

At the end of December 2019, Wuhan city, Hubei province of China, has gathered a global attention due to a sudden and new outbreak of a respiratory disease of unidentified etiology. The source of the outbreak was identified and linked to Huanan Seafood Wholesale Market, where there was a sale of live animals. The clinical presentations were identical to SARS and MERS with initial symptoms of fever, cough, and difficulties in breathing, with progression to severe pneumonia in some patients with underlying morbidity.[4]

STRUCTURE AND COMPOSITION OF CORONAVIRUS

Coronaviruses are large, enveloped single-stranded positive-sense RNA (27–32 kb) viruses measuring around 120–160 nm. The unsegmented genome of coronavirus is the largest genome among all RNA viruses and the isolated genomic RNA is highly infectious. The helical nucleocapsid measures around 9–11 nm in diameter. The outer surface of the envelope has 20-nm-long club or petal-shaped projections that are spaced widely suggestive of a solar corona.[5]

Associated with the viral envelope are three different proteins, which include membrane (M), envelope (E), and spike (S) proteins. The membrane protein (M) and the envelope protein (E) play an important role in virus assembly, whereas the spike protein (S) contributes to virus entry into host cells. Some viruses, including human coronavirus OC43 (HCoV-OC43), encode additional envelope-associated hemagglutinin-esterase (HE) protein that causes hemagglutination and has acetylesterase activity.[6]

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Genomic Nature of Coronavirus

Coronaviruses have the highest frequency of recombination and mutation at a high rate compared to any other positive-strand RNA virus. When a host is infected with multiple coronaviruses, it is capable of combining genetic information from different sources. Following unique nature and properties of this virus, it has become a great challenge for both diagnostic detection as well as therapy (and vaccine) regimens.

The sequence analysis of genome of the present highly pathogenic SARS-CoV-2 revealed that the recognition rates of the complete genome sequence of SARS-CoV and bat SARS coronavirus (SARSr-CoV-RaTG13) were 79.5 and 96%, respectively.[7] It comes under the cluster of beta coronaviruses that includes Bat-SARS-like (SL) ZC45, Bat-SL ZXC21, SARS-CoV, and MERS-CoV. It was studied that phylogeny of the complete RNA-dependent RNA polymerase (RdRp) gene of 2019-nCoV was distinct from SARS-CoV. SARS CoV-2 is now recognized as a novel beta coronavirus that belongs to subgenus Sarbecovirus and suggesting that bats could be the primary source. The fact whether 2019-nCoV is directly transmitted from bats or through an intermediate host is still unknown.

The envelope spike (S) protein plays a primary role in binding to the host cell receptor and membrane fusion, which is a crucial step for determining host tropism and transmission capacity. Earlier studies have uncovered that binding receptors for different coronaviruses are varied, such as SARS-CoV binds to ACE2 and MERS-CoV binds to CD26. The study on molecular modeling showed that there was structural similarity between the receptor-binding domains of SARS-CoV and 2019-nCoV.[8]

Immunopathogenesis of nCoV 2019

The significant determinant factor for the coronavirus is entry into host cells, which is mediated by binding of envelope spike (S) protein to the cellular receptor, ACE2. Upon entry into the host cell, the viral RNA genome from the virus is released into the host cytoplasm, which is subsequently translated into structural proteins and two polyproteins, following which the replication of viral genome begins to occur. Finally, the vesicles containing the virus particles fuse with the host cell membrane to release the virus.

Similar to any viral infections, the antigens are processed and presented by virus-specific B and T cells, which in turn stimulate the body's immune system. Studies on immunological mechanisms have shown that total number of circulating CD4 and CD8 T lymphocytes in the peripheral blood is significantly reduced in patients infected with SARS-CoV-2.

The main cause of death in COVID-19 is found to be acute respiratory distress syndrome (ARDS) as per a report in Lancet.[9] Cytokine storm, which is defined as the uncontrolled systemic inflammatory response, leads to release of large amounts of pro-inflammatory cytokines and chemokines by immune effector cells and is the most important mechanism of ARDS in SARS-CoV infection. This cytokine storm tends to trigger the immune system of the body in an exaggerated manner, which causes ARDS, multiple organ failure, and disseminated intravascular coagulation (DIC), and leads to death in severe COVID-19 infections.

Clinical Features of SARS-CoV-2

The clinical presentation of SARS-CoV-2 resembles that of viral pneumonia and the severity ranges from mild to severe illness. As per the report of the WHO, approximately 80% of patients present with mild, 14% present with severe, and 5% present with critical illness.[10]

The most common routes of transmission of SARS-CoV-2 is by droplets, close contact, and aerosol spread. The incubation period of SARS-CoV-2 ranges from 2–10 days with a median incubation period of 3 days. The study done on clinical presentation following an outbreak in China has shown that the most common symptoms of COVID-19 were fever (87.9%), cough (67.7%), and fatigue (38.1%), with rare presentation of diarrhea (3.7%) and vomiting (5.0%). Most patients had some degree of dyspnea at presentation and severe cases progressed to complications, including ARDS, acute heart injury, and secondary infection.[11]

Laboratory Diagnosis of 2019-nCorona Virus

Sample Collection

Sample collection for COVID-19 should be done by trained personnel with caution and should strictly adhere to Infection Prevention and Control Guidelines considering all samples as potentially infectious. The person who collects and handles the sample should follow standard precautions, which include proper hand hygiene, gown, gloves, facial protection, goggle, face shield, and respirator (N95 or FFP2).

Detection and collection of samples from SARS-CoV-2 suspects was done as per the case definition and guidelines of SARS-CoV-2 infection of the Ministry of Health and Family Welfare.[12]

Samples collected for virus testing include specimens from the upper respiratory tract such as nasopharyngeal swab, oropharyngeal swab, or wash in ambulatory patients and from patients suffering from severe lower respiratory disease specimens like sputum, endotracheal aspirate, or bronchoalveolar lavage. Other specimens that can be collected include biopsy tissue or autopsy specimens from the lungs, whole blood, urine samples, and serum samples (acute and convalescent) for serological testing. Collected specimens were stored at 2–8°C for up to 72 hours after collection. If a delay in processing and testing of samples or shipping is expected, specimens are stored at −70°C or below.

The recommended diagnostic methods for COVID-19 cases include detection of COVID-19 virus nucleic acid (RNA) by real-time RT-PCR assays for routine confirmation. The next step to distinguish the characteristics of 2019-nCoV from other coronaviruses is whole-genome sequencing and genome analysis by bioinformatics including phylogenic tree construction.[13,14] Immunoassays, which are generally lateral flow assays, are rapid point-of-care immunoassays, developed for rapid detection of SARS-CoV-2 antigens or antibodies (IgM and IgG) against COVID-19 infections.

Laboratory Diagnosis of SARS-CoV-2

- Detection of viral RNA: Detection of RdRp(1), RdRp(2), E gene, and N gene from respiratory secretions by real-time RT-PCR
- Detection of serum antibody: Screening for IgM and IgG by ELISA/rapid diagnostic test
- Detection of antigen: Secreted proteins by LFA/ELISA using specific monoclonal antibody.
- Isolation of virus: SARS-CoV-2 can be isolated from respiratory specimens using Vero cell line

Detection of Viral Nucleic Acid (Nucleic Acid Amplification Tests for COVID-19)

Routine diagnostic modality adopted for confirmation of COVID-19 cases is by detection of virus nucleic acid (RNA) by real-time RT-PCR

assays. The targets in the viral genome include the N, E, S, and RdRP genes. All procedures of RNA extraction should always be done in a biosafety cabinet in a BSL-2 or equivalent facility only.

The extracted RNA was immediately used for testing the presence of SARS-CoV-2 using the real-time RT-PCR protocol published by the WHO[15] for the detection of RdRp (1), RdRp (2), E gene, and N gene. RNase P gene was used as the internal control for the analysis. Confirmatory laboratory tests were performed as per the WHO-recommended test protocols.[16] These samples were also sequenced using the NGS approach to retrieve the complete genome of the virus.

False-negative result in an infected person may be due to the poor specimen quality, insufficient patient material, specimen collected very early or in late stage of infection, the sample not appropriately handled and shipped, or could be due to inherent technical reasons in the test, e.g., virus mutation or PCR inhibition.

Demonstration of high viral RNA of SARS-CoV-2 in the fecal material in certain patients with COVID-19 pneumonia proves the enteric involvement in severe novel coronavirus infections. Thus, apart from respiratory sampling in advanced COVID-19 cases, other preferred method for detecting SARS-CoV-2 may be rectal swab by real-time RT-PCR.[17,18]

Serological Testing

Serological testing can help in investigating an extent of ongoing outbreak and also the retrospective assessment of the attack rate. In cases of strong suspect to COVID-19 infection, where nucleic acid amplification test (NAAT) assays are still negative, testing of paired serum samples (samples collected during acute and convalescent phases) could support the diagnosis once validated serology tests are available.[19]

Serological diagnosis is significant in case of mild to moderate ill patients who present with late onset of symptoms, beyond the first 2 weeks of illness. It is also defined as an important tool to identify and understand the extent of COVID-19 spread in the community and to label the individuals who are immune and potentially "protected" from becoming infected.

Antigen detection: Viral proteins (antigens) are generated approximately 1–5 days, after the onset of symptoms. But the mechanism of production and secretion of these proteins has not been established yet. Various methods like ELISA and immunofluorescence are available, but they are less accurate than PCR tests.

Antibody detection: Detection of IgM/IgG antibodies can assist in outbreak investigation and seroprevalence studies. Although several assays such as ELISA and rapid diagnostic tests are available for the detection of IgM /IgG antibodies, these tests are not recommended for use to date. The detection of total antibodies is the most sensitive and earliest serological marker. Its levels will increase from the 2nd week of onset of symptoms.[20]

Isolation of Virus

Viral culture of SARS-CoV-2 should be carried out in a biosafety level-3 facility according to laboratory biosafety guidelines. However, for a routine diagnostic procedure, isolation of SARS CoV-2 virus is not recommended.

Viral Sequencing

Virus whole genome sequencing is an important tool to study the molecular epidemiology. Viral sequencing not only confirms the presence of the virus, regular sequencing of a percentage of clinical specimens helps to monitor viral genome mutations that might affect the performance of medical countermeasures, including diagnostic tests.

INTERPRETATION OF RESULTS OF COVID-19 (BASED ON CDC GUIDANCE)

Nucleic acid test/ viral testing	Antibody testing	Interpretation
Positive	–	Currently suffering from active COVID-19 infection
Negative	–	Do not suffer from an active COVID-19 infection
–	Positive	Had past infection with COVID-19
–	Negative	Never had (or have not yet developed antibodies to) COVID-19 infection
Positive	Positive	Currently have an active COVID-19 infection and can spread the virus to others
Positive	Negative	Currently have an active COVID-19 infection and can give the virus to others
Negative	Positive	Had and recovered from a COVID-19 infection
Negative	Negative	Never had a COVID-19 infection

THERAPEUTIC INTERVENTION FOR COVID-19

In the current scenario, there are no approved treatment or clinically proven antiviral agent specific for SARS-CoV-2 infection. Hence, management strategy for COVID-19 patients is mainly supportive treatment, which includes oxygen therapy, conservative fluid management, and to combat secondary bacterial infection with appropriate broad-spectrum antibiotics. Researches on molecular mechanisms and the genomic organization of SARS-CoV-2 have identified several potential therapeutic targets to combat against this novel coronavirus with the available and existing antiviral agents.

The WHO initiated the "solidarity trial" in several countries that focus to compare the efficacy of the drug regimens such as remdesivir, lopinavir/ritonavir, lopinavir/ritonavir with interferon beta, and hydroxychloroquine against COVID-19. Recently, the US FDA has approved the use of convalescent plasma from patients recovered from COVID-19 for the treatment of severe or life-threatening COVID-19 infections. The Indian Council of Medical Research (ICMR) has already sought participation in a phase-II randomized controlled trial to assess the safety and efficacy of convalescent plasma.

PREVENTION OF COVID-19

The bulletin from the National Task Force for Covid-19 of the ICMR, dated March 21, 2020, recommends the use of hydroxychloroquine for prophylaxis in (i) asymptomatic healthcare workers involved in the care of suspected and confirmed patients and (ii) household contacts of confirmed patients, with the viral infection.[21]

To develop effective vaccine for COVID-19, there are ongoing multiple attempts and to date no vaccine has completed clinical trials. Since COVID-19 is a novel virus, it requires innovative vaccine technologies and development strategies.[22] For COVID-19, at least three vaccination strategies are being investigated. First strategy would be to develop either an inactive or dead whole virus vaccine, which aims to elicit a prompt immune response of the human to a new infection with COVID-19. A second strategy would be a subunit vaccine, that can sensitizes the immune system to certain subunits of the virus. In the case of SARS-CoV-2, the target protein would be the S-spike protein that helps the virus to attach to the ACE2 enzyme receptor. A third strategy, a novel technique, is to develop nucleic acid vaccines (DNA or RNA vaccines). Experimental vaccines developed based on any of these strategies would have to be tested for their safety and efficacy.[23]

As of May, 120 vaccine candidates were in development,[24] of which five having been initiated in phase I–II safety and efficacy studies in human subjects and six in phase I trials. Phase I trials primarily test for safety and preliminary dosing in a few dozen healthy subjects, while phase II trials proceed following success in phase I trials, which evaluate immunogenicity, dose levels, and adverse effects of the candidate vaccine, typically in hundreds of people. While phase I–II trials are typically randomized and placebo-controlled, preliminary aimed at safety and immunogenicity testing, phase III trials typically involve more participants, which include control group, and test effectiveness of the vaccine to prevent the disease and also monitor for adverse effects at the optimal dose.[25,26]

The best practice adopted to prevent illness is to avoid being exposed to this virus by adhering to the practices like regularly and thoroughly cleaning the hands with an alcohol-based hand rub or with soap and water; maintaining at least 1 m (3 feet) distance between people; avoiding going to crowded places; avoiding touching eyes, nose, and mouth; following good respiratory hygiene; staying home and self-isolating if found to have minor symptoms such as cough, headache, and mild fever; and seeking medical attention following difficulty breathing.

CONCLUSION

In conclusion, SARS-CoV-2, which is categorized under contagious respiratory infectious disease, belongs to the Sarbecovirus subgenus of the Coronaviridae family and is the seventh coronavirus known to infect humans. As some cases are life-threatening and cause severe illness, COVID-19 poses a great threat to global health and safety. The specific mechanism of the virus still remains unknown, and no specific antiviral drugs and vaccines have been developed. At present, controlling the spread of the epidemic and reducing mortality can be achieved by strictly adhering to the proactive measures like controlling the source of infection, cutting off the route of transmission, and using existing drugs to control the progress of disease.

REFERENCES

1. World Health Organization. Naming the coronavirus disease (COVID-19) and the virus that causes it. Geneva: WHO; 2020 [Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/naming-the-coronavirus-disease-(covid-2019)-and-the-virus-that-causes-it. (mention the access date).
2. World Health Organization. MERS situation update. 2019. http://applications.emro.who.int/docs/EMROPub_2019_MERA_apr_EN_23513.pdf (accessed on August 20,2019).
3. Situation report by WHO on Novel Coronavirus (2019-nCoV) https://www.who.int/emergencies/diseases/novel-coronavirus-2019.
4. Salata C, Calistri A, Parolin C, Palu G. Coronaviruses: a paradigm of new emerging zoonotic diseases. Pathog Dis 2019;77(9):ftaa006. DOI: 10.1093/femspd/ftaa006.
5. Brooks GF, Janet SB, Morse SA, Jawetz EJ. Melnick & Adelberg's Medical Microbiology. 28th ed., New York, N.Y.: Lange Medical Books/McGraw-Hill, Medical Pub. Division; 2019.
6. Fehr AR, Perlman S. Coronaviruses: an overview of their replication and pathogenesis. Methods Mol Biol 2015;1282:1–23. DOI: 10.1007/978-1-4939-2438-7_1.
7. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 2020;395(10223):507–513. DOI: 10.1016/S0140-6736(20)30211-7.
8. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. The Lancet 2020;;395(10224):565–567. DOI: 10.1016/S0140-6736(20)30251-8.
9. Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Respir Med 2020;8(4):420–422. DOI: 10.1016/S2213-2600(20)30076-X.
10. World Health Organization. Coronavirus disease 2019 (COVID-19) situation report - 40. WHO; 2020.
11. Guan W, Ni Z, Hu Y, Liang W, Ou C, He J, et al. Clinical characteristics of 2019 novel coronavirus infection in China. N Engl J Med 2020;382(18):1708–1720. DOI: 10.1056/NEJMoa2002032.
12. Ministry of Health & Family Welfare, Government of India; 2020. Available from: https://mohfw.gov.in/node/4904, accessed on February 18, 2020.
13. World Health Organization, Laboratory testing for coronavirus disease (COVID-19) in suspected human cases: interim guidance. WHO/COVID-19/laboratory/2020.5. Geneva: WHO; 2020. Available from: https://www.who.int/publications-detail/laboratory-testing-for-2019-novel-coronavirus-in-suspected-human-cases-20200117.
14. Centers for Disease Control and Prevention. CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel, Instructions for Use. Atlanta: CDC; 2020. Available from: https://www.fda.gov/ media/134922/ download.
15. World Health Organization. Coronavirus disease (COVID-2019) situation reports. WHO; 2020. Available from: https://www.who.int/emergencies/diseases/novel coronavirus-2019/situation-reports, accessed on February 29, 2020.
16. World Health Organization. Coronavirus disease (COVID-19) technical guidance: Laboratory testing for 2019-nCoV in humans. Available from: https://www.who.int/emergencies/ diseases/ novel-coronavirus-2019/technical-guidance/laboratory-guidance, accessed on February 29, 2020.
17. Cheng PK, Wong DA, Tong LK, Ip SM, Lo AC, Lau CS, et al. Viral shedding patterns of coronavirus in patients with probable severe acute respiratory syndrome. Lancet 2004;363(9422):1699–1700. DOI: 10.1016/S0140-6736(04)16255-7.
18. Zhang W, Du RH, Li B, Zheng XS, Yang XL, Hu B, et al. Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. Emerg Microbes Infect 2020;9(1):386–389. DOI: 10.1080/22221751.2020.1729071.
19. Sethuraman N, Jeremiah SS, Ryo A. Interpreting diagnostic tests for SARS-CoV-2. JAMA 2020;323(22):2249–2251. DOI: 10.1001/jama.2020.8259.
20. Lou B, Li T, Zheng S, Su Y, Li Z, Liu Wei, et al., Serology characteristics of SARS-CoV-2 infection since the exposure and post symptoms onset. medRxiv. Preprint posted March 27, 2020.
21. National Task Force for Covid 19. Advisory on the use of Hydroxy-chloroquine as prophylaxis for SARS-CoV-2 infection. New Delhi:

ICMR; 2020 March 22 [cited 2020 Apr 14]. Available from: https://icmr.nic.in/ sites/ default/ files/ upload_documents/ HCQ_ Recommendation_22March_final_MM_V2.pdf.

22. Thanh LeT, Andreadakis Z, Kumar A, Gómez Román R, Tollefsen S, Saville M, et al. The COVID-19 vaccine development landscape. Nat Rev Drug Discov 2020;19(5):305–306. DOI: 10.1038/d41573-020-00073-5.

23. Chen WH, Strych U, Hotez PJ, Bottazzi ME. The SARS-CoV-2 vaccine pipeline: an overview. Curr Trop Med Rep 2020(2):1–4. DOI: 10.1007/s40475-020-00201-6.

24. Update on WHO Solidarity Trial – Accelerating a safe and effective COVID-19 vaccine". World Health Organization. 27 April 2020. accessed on 2 May 2020.

25. Vaccine Safety - Vaccines" (https://www.vaccines.gov/basics/safety). www.vaccines.gov. US Department of Health and Human Services. accessed on 13 April 2020.

26. "The drug development process" (https://www.fda.gov/patients/learn-about-drug-and-device-approvals/drug-development-process). US Food and Drug Administration. 4 January 2018. accessed on 12 April 2020.