Insight into Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2): Rationalized Review Special Reference to COVID-19

Bedanta Bhattacharjee¹, Abu Md Ashif Ikbal^{2*}, Ram Kumar Sahu², Meenakshi Ratra³

¹Department of Pharmaceutical Sciences, Faculty of Science and Engineering, Dibrugarh University, Dibrugarh – 786004, Assam, India

²Department of Pharmaceutical Science, Assam University (A Central University), Silchar-788011, Assam, India

³VMS College of Pharmacy, Batala-143505, Punjab, India

ABSTRACT

The Coronavirus disease (COVID-19) is a worldwide pandemic caused by Severe acute respiratory syndrome 2 (SARS-CoV-2) which make an appearance in Wuhan China and spread globally. Coronavirus is a highly contagious virus, under the family Coronaviridae. SARS-CoV-2 causes respiratory breathing problems and can be transmitted through close contact, respiratory droplets and aerosol transmission. The binding of the structural spike proteins with the human ACE2 receptors plays a key role to initiate the event and severe complication of the disease. Clinical manifestation includes high fever, cough and dyspnoea. At present, there is no specific therapeutic strategy is available against the virus, only supportive care and management may be provided to the patients. In this review, we mainly focus and analyze the genetic morphology, pathogenesis and the therapeutic intervention. We further discuss the future perspective to confront with this viral epidemic.

Keywords: SARS-CoV-2, Pandemic, Coronaviridae, ACE2 receptors

Article History:

Received: 17 October 2020

Revised: 20 Dec 2020

Accepted: 22 Dec 2020

Published: 1 Jan 2021

Corresponding Author:

Abu Md Ashif Ikbal, Department of Pharmaceutical Science, Assam University (A Central University), Silchar-788011, Assam, India

Email: abum97@gmail.com

Mobile: +917002666727

INTRODUCTION

The COVID-19 pandemic is real. The outbreak of the virus has proved to be a life threat to humans. At the end of December 2019, Wuhan city of Hubei province in central China, allestation severe cases of pneumonia of unknown origin. As of June 2020, there were 10.3M confirmed cases across worldwide with 506K death¹. On 7 January 2020, a human sample from the throat swab of a patient identified the novel coronavirus by Chinese authorities². Later, the pathogen was renamed as SARS-CoV-2 and the disease as coronaviruses disease 2019³. On 30

January 2020, WHO declared the SARS-CoV-2 outbreak as an Public Health Emergency of International Concern (PHEIC)⁴. The causative virus belonged to the family coronaviridae, a member of the β group of coronavirus. In 2003, over 26 countries reeled under SARS CoV infected 8098 people with rate of mortality 9 %. However, till the date of this writing, the novel coronavirus 2019 infected 10.3M people with mortality rate of 4.8% over 109 countries. It showed that the spreading rate of SARS – CoV-2 seemed to be greater than SARS-

CoV⁵⁻⁷. This review makes use of combing research article, multiple origin & social media posts. In this review article, we briefly summarize the genetic morphological structure, pathogenesis, mode of transmission, treatment strategy, laboratory diagnosis as well as future perspective.

GENETIC MORPHOLOGY OF CORONAVIRUS

This virus is also very similar to the previous SARS Coronavirus and is therefore named as SARS-CoV-2. SARS-CoV-2 is a helical symmetry, positive sense single stranded large RNA viruses under the family Coronaviridae, order Nidovirales and enveloped in a lipid bilayer membrane, sensitive to lipid solvent, having genome size of approximately 30kb⁸. There are four major genera of Coronavirus and are categorized into – the alpha and beta – which originated from rodents and bats, gamma and delta coronavirus – which originated from avian species. SARS-CoV-2 belongs to the genus β – coronavirus. The first two groups of coronaviruses primarily infect mammals whereas the latter two groups mainly infects birds⁹⁻¹¹. In the recent study, it has been shown that Angiotensin Converting Enzyme 2 receptor expressed by lung epithelial cells, kidney, blood vessels is used by SARS-CoV-2 to gain entry into the human cells¹². The Coronavirus virions contains several structural proteins including the Spike protein (S), the Transmembrane protein (M), the Envelope protein (E), the Nucleocapsid protein (N), few coronaviruses contain the Hemagglutinin Esterase (HE) and several non-structural proteins (nsp) are also encoded by it (Fig 1). Both Transmembrane protein M & Envelope protein E are involved in virus gathering processes⁹. The N protein is responsible for the formation of nucleocapsid by coating RNA, necessary for RNA synthesis and it binds to the CoV genome and soluble in nature. It also plays a crucial role in the viral RNA replication process and cellular response to viral infection against the host. Expression of N protein increase the production of virus like particles as shown by a recent study¹³. The S glycoprotein of the coronavirus is responsible for the petal shaped or crown like projection on their surface from which the name “Coronavirus” was coined, is found in the virion envelope^{11,13}. Furin like proteases cleaves the S protein into two different functional domain viz S1 – amino and S2 – carboxy terminal. The N terminal S1 domain is involved in receptor binding function whereas C terminal S2

transmembrane protein are involved in viral entry¹⁴. S1 Spike protein primarily help the virion to attach with the Peptidase domain of host receptor (ACE2) and internalization of the virus inside the host cell induces conformational change in the S glycoprotein. S2 Spike proteins involve in the process of fusion of the virion and cellular membrane. The S protein is the main inducer of neutralizing Antibody^{14,16}. The most plentiful structural protein is the membrane glycoprotein responsible for helping the formation of new virus particles by joining virus & host factors. However, in Reverse genetic studies it has been shown that by interacting M protein with the viral ribonucleoprotein and glycoprotein it generates a bridge of M-M interaction having capability to keep out some host membrane proteins from the viral site¹⁷.

PATHOGENESIS

The exact mechanism by which SARS-CoV-2 developed COVID-19 is not known yet. Severely infected Covid-19 patients showed high level of leukocytes counts, respiratory problems, high level of plasma proinflammatory cytokines. In a case report of covid-19, it was seen that a patient developed high body temperature along with cough at 5 days having breathing problems in both lungs of covid-19 patients²⁰. RT-PCR showed positive result from patient sputum sample that confirmed the presence of nucleic acid of SARS-CoV-2²⁰. The laboratory finding revealed that, a blood C- reactive protein level was higher (16.6 mg/L) than the normal range (0- 10 mg/L). Also, D- dimer and erythrocyte sedimentation rate was also markup in these patients²¹. The main primary mechanism of covid-19 pathogenesis was severe pneumonia but there are some other complication like anemia, acute cardiac injury and incidence of ground glass opacities have been identified in subpleural regions of both lungs that leads to death. However, significantly high up blood level of chemokines & cytokines including IL-1 Ra, IL-7, IL-8, IL-9, IL-10, IL-1 β , GCSF, basic FGF2, IP-10, MCP1, TNF α , VEGF-A is also associated with covid-19 patients²¹. High level of proinflammatory cytokines including IL-2, IL-7, IL-10, MCP1, MIP -1 α , GCSF & TNF α upraised in patients with severe cases admitted in ICU²². Angiotensin Converting Enzyme 2 (ACE2) play a key role to initiate the process of pathogenesis of covid-19 infection . SARS-CoV-2 utilized ACE2 as a host receptor. The S glycoprotein of the coronavirus can bind to the ACE2 receptor which are responsible

for the entry of virus into the host cells^{23,24}. S glycoprotein consists of two subunits viz S1 and S2²⁵. After binding to ACE2 receptors, infection generally starts with cells of the respiratory mucosa and then spread to epithelial cells of alveoli in the lungs. Receptor binding causes conformational changes in the S protein followed by fusion of viral membrane with host cell membrane with the help of serine protease like TMPRSS2 or Cathepsin which results in release of the viral genome (made up of RNA) and nucleocapsid protein inside the cells. Then, the virus uses the host cell machinery to replicates its genome, producing viral RNA and proteins^{19,26}. Finally, viral protein, genomic RNA,

nucleocapsid protein merge together to produce a new copy of the virus particles called mature virion in the cytoplasm and then transported via golgi vesicles & they break out from the cell through the process exocytosis and the host cell dies (Fig 2)^{27, 28}. Rapid fast growth of the virus slowly destroyed tissue, producing symptoms. Inflammatory response is triggered by infection, which assembled the immune cells to the site to fight against the virus²⁶. Inflammation is an important defense mechanism, it may become enormous causes damaged to the own body tissues leads to the severity of the disease^{25, 28}.

Fig 1: Structure of Coronavirus

Fig 2: Stages of Life events of SARS-CoV-2 inside the host cells

TRANSMISSION

The first positive COVID-19 cases were reported in Wuhan, China linked to a wet animals wholesale seafood market suggested that SARS-CoV-2 was

transfer from animals to human²⁹. The rapid spread of COVID-19 infection is from aerosol droplets, respiratory droplets, close contacts & fomites³⁰. The mean incubation period was 3-9 days with a range between 0-24 days^{26, 30}. When a covid-19 person

coughs or sneezes, transmission is possible through respiratory droplets. When the person sneezes, the droplets come out and get mixed with air to form an aerosol. Once inhaled it may cause infection²³. A recent study showed that ocular surface having high potential to carry out the highly pathogenic SARS-CoV-2 infection³¹. Other modes of transmission include hand shaking with infected person. If anyone comes in contact with infected person and then repeatedly touches the mouth, nose, eyes there might be a possible chance of transmission³². In a hospital study of 138 covid-19 patients, 41% cases are associated with hospital associated transmission of SARS-CoV-2³³.

In retrospective study it was found and confirmed that there is no possible way of transmission in third trimester covid-19 pregnant patients from mother to baby. Even though pregnant women underwent cesarean section, so there is a possibility of transmission during vaginal delivery was yet to be predicted. As a result pregnant women are exposed to severe respiratory infection³⁴. However, another study of 425 patients found that the ratio of SARS-CoV-2 infection gradually increased in healthcare workers²⁹. Outside China, as of June 30, 2020 there were 591,001 confirmed cases reported in India of which the first case was reported in Thrissur kerala on January 30^{33, 35}.

CLINICAL SYMPTOM SPECTRUM

The observation clinical symptoms of covid-19 infection is very essential, the symptoms are non specific & the most common symptoms includes dry cough, dyspnoea, fever, fatigue & myalgia^{24, 33, 36}. Severe rapid organ dysfunction have been reported such as shock, acute ARDS, arrhythmia, acute cardiac injury & even death³⁷. A study on 99 patients showed that 11% patients died of rapid organ failure & 17% patients developed acute respiratory distress syndrome³⁷.

Pregnant & non pregnant women should have almost identical characteristics^{31, 38}. Gastrointestinal problems like diarrhoea have been reported in corona infected patients^{39, 40}. Although symptoms like sneezing, sore throat and rhinorrhea are associated with covid-19 as it target the lower airway respiratory tract^{22, 41-43}.

LABORATORY DIAGNOSIS

Due to limited number of testing kits, well established laboratory many developing countries as well as in low income countries it will be quite tough for them to arrange all this facilities. Presently, available diagnostics tools for infected patients in laboratory include RT-PCR to confirm the presence of nucleic acid of SARS-CoV-2 in sputum, naso & oropharyngeal swabs, isolation of virus from human clinical specimen (viral cell culture technique), perform certain immunological assay for the detection of antibody & antigen include ELISA, rapid immune chromatographic test etc. So, these methods are very expensive and time consuming^{9, 13, 44, 45, 46}. A positive test generally indicate the presence of covid-19, although sometimes false positive test are also generated because of the mutation in the genome of SARS-CoV-2. If initial testing show negative results but the apprehensive remains for covid-19, then recommended WHO guideline testing procedure from different respiratory tract^{47, 48}. Some other laboratory test may include analysis of CD⁴⁺ & CD⁸⁺ count by flow cytometry, serological biochemistry, chest radiological finding (pneumonia), total blood picture (lymphopenia)³³. Sometimes, coinfection with more than one virus has been noted & have an influence on management decision^{49, 50}.

COMPLICATIONS

Laboratory data mark common peculiarity among covid-19 patients including high level of lactate dehydrogenase, high level of alanine aminotransferase, high level of D-dimer, high level of neutrophils, eosinopenia, lymphopenia, high level of CRP⁵¹⁻⁵⁴. The eosinopenia is associated with covid-19 patients while its sensitivity & specificity are low. Both lymphopenia & eosinopenia change the sensitivity and specific pattern⁵⁴. COVID-19 patients have significantly high level of TNF α , VEGF A, MCP-1, GCSF²¹. High level of troponin indicate infiltration of cardiac tissue⁵⁵. Table 1 exhibit the most frequent finding are eosinopenia & lymphopenia with 78.8% and 68.7%.

Elevated levels of Urea and Cystatin-C in severe COVID-19 patients causes acute kidney injury⁵⁶⁻⁵⁹. There are two principles behind the causes of acute kidney injury. The first theory is from production of more ACE2 levels in the proximal convoluted tubules than the lungs or heart. The second theory related to cytokine storm⁵⁹. Elevated levels of Troponin is encountered in 17.3% patients

associated with ventricular tachycardia, malignant arrhythmia^{55, 60, 61}. Most of the comorbidity associated with COVID-19 is hypertension (30.7%), then Diabetes mellitus (14.3%) followed by Cardiovascular disease (11.9%). Heart failure is also seen with elevated levels of N-terminal pro B type natriuretic and troponin levels, mainly in severe associated cases⁶². A recent published study showed high up level of troponin in severe cases⁶³.

Table 1: COVID-19 Laboratory detection data

Laboratory Detection	Rate (%)
Eosinopenia	78.8
Lymphopenia	68.7
Elevated AST	63.4
Elevated CRP	60.7
Elevated PT	58.0
Elevated LDH	47.2
Elevated D-dimer	46.4
Thrombocytopenia	36.2
Elevated ALT	21.3
Elevated HS Troponin	12.5

LDH – Lactate Dehydrogenase; AST – Aspartate Aminotransferase; PT- Prothrombin time; CRP – C- reactive protein; HS- Troponin – High – sensitivity Troponin

POTENTIAL STRATEGIES

Currently, there is no primary standard care for covid-19. However, research studies are still going on to develop vaccines against covid-19. Development of vaccines takes a long time and it will require several clinical evaluations⁶⁴. The only available therapeutic option mainly focused on symptomatic and supportive care of covid-19 patients as per the guideline. At present covid-19 patient is being treated with antiviral drugs like lopinavir and ritonavir (400mg twice each day and 100 mg twice every day) and Arbidol (200mg two times every day) is suggested but the clinical effectiveness has not been investigated properly yet⁴⁵. The researchers considered the S protein as

a target molecule for developing drugs. Oral and intravenous administration of quinolones and cephalosporins antibiotics is given if fever lasted for more than 7 days or elevated level of C-reactive protein i.e. 30mg/L or more (normal range: 0-8mg/L)⁴⁵. In the United State, a study demonstrated that Remdesivir being effective in treating covid-19 patients and show good invitro results²⁷.

Another study shown, combination of both Remdesivir and Chloroquine antagonizing the in vitro replication process of SARS-CoV-2³³. A study from South Korea, upon administration of lopinavir/ritonavir treatment showed notably reduced SARS-CoV-2 infection in covid-19 patients⁶². Assessment on the efficiency and safety of Lopinavir and Ritonavir in covid-19 patient is still ongoing⁶⁴. In most of the patients, combination of both Lopinavir-Ritonavir effectively clearing the viral load. Ritonavir inhibit the CYP3A4 while this enzyme inactivates Lopinavir and achilles tendinopathy has been reported while taking above combination^{65, 66}. High dose of Vit-C has also been recommended to treat covid-19⁶⁷. According to several published studies, Convalescent Plasma Therapy are suggested for the treatment of covid-19. Administration of convalescent plasma from infected recovered patients showed encouraging promising results⁶⁸. Possible risk factors associated with plasma therapy – Anaphylactic Reactions, Acute lung injury, RBC alloimmunization, Hemolytic transfusion reaction⁶⁹. During treatment, care should be taken to minimize the chance of transmission and reduce complication⁷⁰. Now it is very clear that more research is required to identify the drug for treating of covid-19 infection⁷¹. Griffithsin, an inhibitors of spike glycoproteins have been reported to bind to SARS-CoV-2 glycoproteins by inhibiting the interaction with ACE2 receptor. Recently, India has successfully developed a potential indigenous vaccine candidate name "COVAXIN" in alliance with the Indian Council of Medical Research – National Institute of Virology. The DCGI has been given permission to conduct phase 1 and 2 human clinical trial⁷².

FUTURE PERSPECTIVE

Mostly countries like China, India, Italy and USA as a result of pandemic 2019 it is seen that they are in declining state in several aspects like health, social and economic sectors. The developed countries already facing this pandemic seems to face a

catastrophic perspective where as in low income countries it will be quite tough for them to afford this viral emergency and the consequences will be catastrophic²⁹. In 2003, similarly the global outbreak of SARS-CoV, also the covid-19 will not affect Africa or south America on a large scale suggesting that respiratory viruses spread more effectively in the winter and therefore the southern hemisphere will be affected later. This can also act different according to climate differences, effects of UV on the survival of viruses, immunological differences etc⁷³. By maintaining the hygiene and taking the preventive measures we can lower the rate of spread. To fight the current pandemic situation requires certain steps to reduce the transmission rate from person to person. Special need should be given to protect the elderly patients, infants, medical staff etc⁷⁴. Most of the countries like US, India, UAE have executed country wise lockdown and proper travel screening as a preventive measure to minimize the transmission. Till now, a number of things remain unclear, focused or questioned. As far now, only few pediatric cases have been reported may be because of lack of insufficient testing³⁹.

CONCLUSION

COVID-19 has taken many lives of human and the death count still continues. Everyday the death toll is rising in rapid pace because no specific treatment is available for this disease, so only preventive measures is there to follow the guidelines recommended by government. Several vaccines are under development. All the scientist, researchers, pharmaceutical companies are working day and night to find out possible medication for the cure and we can hope that they will succeed soon. Doctors, paramedics staffs are trying their level best to give comfort to the patients in hospital. For now, they are our savior. The most practical yet simple way to fight the pandemic is to maintain a hygienic life and maintaining social distancing. Government is also trying its best to keep us safe. For that they are providing various guidelines. So as a good and responsible citizen of India we should follow the guidelines to keep ourselves, our family and our country safe. Let us hope for the best that the pandemic ends soon and we are able to get back to our normal life.

FINANCIAL SUPPORT AND SPONSORSHIP

Nil.

CONFLICT OF INTEREST

There are no conflicts of interest.

AUTHOR'S CONTRIBUTIONS

BB and AMAI collected data and drafted the paper under supervision of RKS. All authors read and approved the paper for publication.

REFERENCES

1. Xu B, Kraemer MUG. Open COVID-19 Data Curation Group. Open access epidemiological data from the COVID-19 outbreak. *Lancet Infect Dis*. 2020; 20(5):534-536.
2. Hui DS, Madani TA, Ntoumi F, Kock R, Dar O. The continuing 2019-nCoV epidemic threat of novel coronaviruses to global health-the latest 2019 novel coronavirus outbreak in Wuhan China. *Int J Infect Dis*. 2020; 91: 264 -266.
3. Gorbalenya AEA. Severe acute respiratory syndrome-related coronavirus: The species and its viruses-a statement of the Coronavirus Study Group. *BioRxiv*. 2020; 1-15.
4. Burki TK. Coronavirus in China. *Lancet Respir Med*. 2020; 8(3):238.
5. Cui J, Li F, Shi ZL. Origin and Evolution of Pathogenic Coronaviruses. *Nat Rev Microbiol*. 2019; 17(3): 181-92.
6. Lai CC, Shih TP, Ko WC, Tang HJ, Hsueh PR. SARS-CoV-2 and COVID-19. The Epidemic and The Challenge . *Int J Antimicrob Agents*. 2020; 55(3): 105924.
7. World Health Organization. Laboratory testing for COVID-19 in suspected human cases. : interim guidance, 2 March 2020. World Health Organization. <https://apps.who.int/iris/handle/10665/331329>
8. National Centre for Biotechnology Information. Severe acute respiratory syndrome coronavirus 2 reference genome. 2020.

9. Weiss SR, Leibowitz JL. Coronavirus pathogenesis. *Adv Virus Res.* 2011; 81: 85-164.
10. Li F. Structure, function and evolution of coronavirus spike proteins. *Annu Rev Virol.* 2016; 3: 237-261.
11. Schwartz DA, Graham AL. Potential maternal and infant outcomes from (Wuhan) coronavirus 2019-nCoV infecting pregnant women: Lessons from SARS, MERS, and other human coronavirus infections. *Viruses.* 2020; 12: 194.
12. Zhou P, Yang XL, Wang XG. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020; 579: 270-273.
13. Chen Y, Liu Q, Guo D. Emerging coronaviruses. Genome structure, replication, and pathogenesis. *Journal of Medical Virology.* 2020; 92(4): 418-423.
14. Hasoksuz M, Sreevatson S, Cho KO, Hoet AE, Saif LJ. Molecular analysis of the S1 subunit of the spike glycoprotein of respiratory and enteric bovine coronavirus isolates. *Virus Research* 2002; 84(1-2): 101-109.
15. Hasoksuz M, Lathrop S, Al-Dubaib MA, Lewis P, Saif LJ. Antigenic variation among bovine enteric coronaviruses (BECV) and bovine respiratory coronaviruses (BRCV) detected using monoclonal using antibodies. *Archives of Virology.* 1999; 144 (12): 2441-2447.
16. Bosch BJ, Van Der Zee R, De Haan CAM, Rottier PJM. The Coronavirus Spike Protein is a class virus fusion Protein: Structural and Functional Characterization of the fusion core complex. *Journal of Virology* 2003; 77 (16): 8801-8811.
17. Neuman BW, Kiss G, Kunding AH, Bhella D, Baksh FM. A structural analysis of M protein in coronavirus assembly and morphology. *Journal of Structural Biology.* 2011; 174(1): 11-22.
18. Schoeman D, Fielding BC. Coronavirus envelope protein. *Current knowledge . Virology Journal* 2019; 160(69): 2-22.
19. Satija N, Lal SK. The molecular biology of SARS coronavirus. *Annals of the New York Academy of Sciences* 2007; 1102: 26-38.
20. Lei J, Li J, Qi X. CT imaging of the 2019 novel coronavirus (2019-nCoV) pneumonia, *Radiology.* 2020; 295(1):18.
21. Huang C, Wang Y, Li X, Ren L, Zhao J. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet.* 2020; 395: 497-506.
22. Wang W, Tang J, Wei F. Updated understanding of the outbreak of 2019 novel coronavirus in Wuhan, China. *J Med Virol.* 2020; 9(4): 441-447.
23. Guo YR, Cao QD, Hong ZS, Tan YY, Chen SD, Wang DY. The origin, transmission and clinical therapies on coronavirus disease 2019 outbreak – an update on the status. *Med Res.* 2020; 7(1): 11.
24. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The Lancet.* 2020a; 395: 497-506.
25. Zhang CY, Jiang S. Adaptive evolution of the spike gene of SARS coronavirus: changes in positively selected sites in different epidemic groups. *BMC Microbiol.* 2006; 6: 88.
26. de Wilde AH, Snijder EJ, Kikkert M, Van Hemert MJ. Host factors in coronavirus replication. *Curr Top Microbiol Immunol* 2018; 419: 1-42.
27. Hussain S, Chen Y, Yang Y, Xu J, Peng Y, Wu Y, Li Z, Guo D. Identification of subgenomic RNAs and noncanonical transcription initiation signals of severe acute respiratory syndrome coronavirus. *J Virol.* 2020; 79: 5288-5295.
28. Sawicki SG, Sawicki DL. Coronavirus transcription. A perspective. *Curr Top Microbiol Immunol.* 2020; 287: 31-55.
29. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y. Early transmission dynamics in wuhan, China, of novel coronavirus-infected

- pneumonia. *N Engl J Med.* 2020; 382(13): 1199-207.
30. Carlos WG, Dela C, Cao B, Pasnick S. (2019-nCoV) coronavirus. *Am J Respir Crit Care Med.* 2020; 201(4): 7-8.
 31. Lu H, Stratton CW, Tang YW. Outbreak of pneumonia of unknown etiology in wuhan , china : The mystery and the miracle. *J Med Virol.* 2020; 92: 401-402.
 32. Chan JFW, Yuan S, Kok KH, Chu H, Yang J, Xing F, Liu J, Hui CKM, Yuen KY. Afamiliar cluster of pneumonia associated with the 2019 novel coronavirus indicating person to person transmission: A study of a family cluster. *The Lancet.* 2020; 395: 514-523.
 33. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus – infected pneumonia in wuhan, china. *JAMA* 2020; 323(11):1061-9.
 34. Jaimes JA, Millet JK, Stout AE, Andre NM, Whittaker GR. A tale of two viruses: The distinct spike glycoproteins of feline coronaviruses. *Viruses.* 2020; 12(1):83.
 35. WHO: Coronavirus disease 2019 (COVID-19) situation report – 23. Geneva, Switzerland: World Health Organization 2020.
 36. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med.* 2020; 382(8): 727-733.
 37. Chen N, Zhou M, Dong X, Gong F. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: A descriptive study. *Lancet.* 2020; 395: 507–13.
 38. Loe M, Tang YW. Laboratory diagnosis of emerging human coronavirus infection. The state of the art. *Emerg Microbes Infect.* 2020; 9: 747-756.
 39. Assiri A, Al-Tawfiq JA, Al-Rabeeah AA, Al-Hajjar S, Al-Barrak A. Epidemiological, demographic, and clinical characteristics of 47 cases of Middle East respiratory syndrome coronavirus disease from Saudi Arabia: a descriptive study. *Lancet Infect Dis.* 2013; 13: 752-61.
 40. Lee N, Hui D, Wu A, Chan P, Cameron P, Joynt GM. A major outbreak of severe acute respiratory syndrome in Hong Kong. *N Engl J Med.* 2003; 348: 1986-94.
 41. Phan LT, Nguyen TV, Luong QC. Importation and human to human transmission of a novel coronavirus in Vietnam. *N Engl J Med.* 2020; 382(9):872-874.
 42. Pal M. Severe acute respirsatory syndrome. A newly recognized viral zoonosis of public health concern. *Acta Scientific Microbiology.* 2018; 1(6): 31-42.
 43. Xu XW, Wu .X, Jiang XG. Clinical findings in a group of patients infected with the 2019 novel coronavirus (SARS-CoV-2) outside of Wuhan, China: retrospective case series. *BMJ.* 2020; 368: m606.
 44. Nie QH, Luo XD, Hui WL. Advances in clinical diagnosis and treatmentof severe acute respiratory syndrome. *World J Gastroenterol.* 2003; 9: 1139-1143.
 45. Guan M, Chan KH, Peris JS. Evaluation and validation of an enzyme- linked immunosorbent assay and an immunochromatographic test for serological diagnosis of severe acute respiratory syndrome. *Clin Diagn Lab Immunol* 2004; 11: 699-703.
 46. Chan PK, To WK, Ng KC. Laboratory diagnosis of SARS. *Emerg Infect Dis.* 2004; 10: 825-831.
 47. Ling Y, Xu SB, Lin YX, Tian D, Zhu ZQ, Dai FH, Wu F, Song ZG. Persistence and clearance of viral RNA IN 2019 novel coronavirus disease rehabilitation patients. *Chin Med J.* 2020; 133(9):1039-1043.
 48. Lim J, Jeon S, Shin HY, Kim MJ, Seong YM, Lee WJ, Choe KW. Case of the index patient who caused tertiary transmission of COVID-19 infection in Korea: The application of Lopinavir/Ritonavir for the treatment of COVID-19 infected pneumonia monitored by

- quantitative RT-PCR. *J Korean Med Sci.* 2020; 35.
49. Guan WJ, Ni ZY, Hu Y. Clinical characteristics of 2019 novel corona virus infection in china. *N Engl J Med.* 2020; 35(6): e79.
 50. Ma C, Gu J, Hou P. Incidence, clinical characteristics and prognostics factor of patients with COVID-19 : a systematic review and meta-analysis. *Med Rxiv.* 2020.
 51. Bai T, Tu S, Wei Y. Clinical and laboratory factors predicting the prognosis of patients with COVID-19: analysis of 127 patients in wuhan, china. *Lancet* 2020. <https://doi.org/10.1101/2020.03.20.20037325>.
 52. Li Q, Ding X, Xia G. A simple laboratory parameter facilitates early identification of COVID-19 patient. *Med Rxiv.* 2020.
 53. Driggin E, Madhavan MV, Bikdeh B. Cardiovascular consideration for patient, health care works, and healthsystems during the coronavirus disease 2019 (COVID-19) pandemic. *J Am Coll Cardiol* 2020; 75(18): 2352-2371.
 54. Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S. Risk factors associated with acute respiratory distress syndrome and death in patient with coronavirus disease 2019 pneumonia in wuhan, china. *JAMA Intern Med* 2020; 180(7):934-943.
 55. Liu Y, Sun W, Li J. Clinical features and progression of acute respiratory distress syndrome in coronavirus disease 2019. *Med Rxiv.* 2020.
 56. Yang XH, Sun RH, Chen DC. Daignosis and treatment of COVID-19: acute kidney injury cannot be ignored. *Zhoughua Yi Xue Za Zhi.* 2020; **100**(16):1205-1208.
 57. Xu D, Zhang H, Gong HY. Identification of a potential mechanism of acute kidney injury during the COVID-19 outbreak: a study based on single – cell- transcriptome analysis. *Preprints.* 2020; 46(6):1114-1116.
 58. Tan ZC, Fu LH, Wang DD, Hong K. Cardiac manifestation of patients with COVID-19 pneumonia and related treatment recommendation. *Zhonghua Xin Xue Guan Bing Za Zhi.* 2020; 48: e005.
 59. Guo T, Fan Y, Chen M. Cardiovascular implication of fatal outcomes of patient with coronavirus disease 2019 (COVID-19). *JAMA Cardiol.* 2020; 5(7):811-818.
 60. Chen C, Yan JT, Zhou N, Zhao JP, Wang DW. Analysis of myocardial injury in patients with COVID-19 and association between concomitant cardiovascular disease and severity of COVID-19. *Zhonghua Xin Xue Guan Bing Za Zhi* 2020; 48: e008.
 61. Lippi G, Lavie CJ, Sanchis-Gomar F. Cardiac troponin 1 in patient with coronavirus disease 2019 (COVID-19): evidence from a meta-analysis. *Prog Cardiovasc Dis.* 2020; 63(3):390-391.
 62. Chen D, Xu W, Lei Z, Huang Z, Liu J, Gao Z. Recurrence of positive SARS-CoV-2 RNA in COVID-19: A case report. *Int J Infect Dis.* 2020; 93: 297-299.
 63. Hull MW, Montaner JSG. Ritonavir – boosted protease inhibitors in HIV therapy. *Ann Med.* 2011; 4(5): 375-388.
 64. Park SY, Lee JS, Son JS, Ko JH, Peck KR, Jung Y. Post exposure prophylaxis for Middle East respiratory syndrome in healthcare workers. *J Hosp Infect.* 2019; 101(1): 42-46.
 65. Matthay MA, Aldrich JM, Gotts JE. Treatment for severe acute respiratory distress syndrome COVID-19. *Lancet Respir Med.* 2020; 8(5):433-434.
 66. Duan K, Liu B, Li C, Zhang H, Yu T, Qu J, Zhou M, Chen L, Meng S, Hu Y, Peng C, Yuan M, Huang J, Wang Z, Yu J, Gao X, Wang D, Yu X, Li L, Li B, Xu Y, Peng Y, Hu Y, Lin L, Liu X, Yu D, Hou J, Shi Z. Effectiveness of convalescent plasma therapy in severe COVID-19 patients. *Proc Natl Acad Sci USA.* 2020; 117(17): 9490-9496.

67. Pandey S, Vyas GN. Adverse effects of plasma transfusion. *Transfusion*. 2012; 52(1): 65-79.
68. Lau AC, Yam LY, So LK. Management of critically ill patients with severe acute respiratory syndrome (SARS). *Int J Med Sci*. 2004; 1: 1-10.
69. Toots M, Cao R, Cox RM, Hart M, Sticher ZM. Characterization of orally efficacious influenza drug with high resistance barrier in ferrets and human airways epithelia. *Sci Transl Med*. 2019; 11.
70. <https://www.timesnownews.com/health/article/india-s-first-covid-19-vaccine-covaxin-gets-dcgi-approval-for-clinical-trials/613790> .
71. El Bcheraoui C, Mimche H, Miangotar Y, Krish V.S, Ziegeweid F, Krohn KJ, Ekat MH, Olsen HE. Burden of disease in Africa, 1990-2017: A systematic analysis for the global burden of disease study 2017. *Lancet Glob. Health* 2020; 8: 341-351.
72. Jin YH, Cai L, Cheng ZS, Deng T, Fan YP. A rapid advice guideline for the diagnosis and treatment of 2019 novel coronavirus (2019-nCov) infected pneumonia (standard version). *Mil Med Res*. 2020; 7(1): 4.