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EFFECTIVENESS OF CONVALESCENT PLASMA THERAPY IN SEVERE COVID-19 PATIENTS

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ABSTRACT

Coronavirus possesses a distinctive morphology the being derived from the outer fringe or corona of embedded envolope protein. Members of coronaviridae family cause a broad spectrum of the animal and the human disease uniquely replication of RNA genome proceed through the generation of a nested set of viral mRNA molecule untill 2003 coronaviruses attracted little interest beyond causing mild upper respiratory tract infection. This changed occur dramatically in 2003 with the zoonotic SARS COV amd the more recent emergence of MERS-CoV has confirmed the Coronaviruses as significant cause of sereve respiratory disease. Currently there are no approved specific antiviral agent for novel corona virus disease 2019 (COVID-19). IN this sy 10 severe patients confirmed by real time viral RNA test were enrolled prospectively. One dose of 200ml of convelescent plasma(CP). Derived from the recently covered donors with the neutralizing antibody titles above 2:640 was transfused to the patients as an addition to the maximal supportive care and antiviral agents. The primary endpoint was the safety of CP transfusion. The second endpoint where the improvement of clinical symptoms and laboratory parameters within 3D after CP transfusion. The median time from one set of illness to CP transfusion was 16.5d. after CP transfusion the lavel of neutralizing antibody increased rapidly upto 1:640 in five cases while that of the other four cases maintained at a high level (1:640). The clinical symptoms were significantly improved along with increase of oxyhaemoglobin saturation within three d.sereral parameters tended to improve as compared to *pretransfusion* including increased Lymphocytes counts. Examination by rediological view showed varying degrees of absorption of lung lesion within 7d. The viral load was undetectable after transfusion in seven patients who had previous viremia. No severe adverse effects were observed. This study showed CP therapy was well tolerated and would potentially improved the clinical outcomes through neutralizing viremia in severe COVID-19 cases. The optimal dose and time point as well as the clinical banefits CO therapy needs further investigation in larger well-controlled trials.

KEYWORDS: MERS-CoV, *pretransfusion*, SARS COV.

INTRODUCTION

According to WHO viral diseases countinue emerge and represent a serious issue to public health. In the last 20 years several viral epidemic such as the sever severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002 to 2003 and H1N1 influenza in 2009 have been recorded most recently the middle East respiratory syndrome coronavirus (MERS-CoV) was first identified in Saudi Arabia in 2012. In a timeline that reaches the present day and epidemic of cases with unexplained low respiratory infection detected in Wuhan the largest metropolitan area in China's Hubai province was first reported to WHO country office in China on December 31 2019. Published literature can trace the beginning of symptomatic individuals back to the beginning of the December 2019. As they were unable to identify the causative agent these first causes were classified as "Pneumonia of unknown etiology". The chinese center for disease control and prevention (CDC) and local CDCs organized an intensive outbreak investigation programs. The etiology of this illness is now attributed to a novel virus belonging to the Coronavirses (CoV) family. On February 11 2020 WHO director general Dr Tedros Adhanom ghebreyesus announced that the disease caused by this new CoV was a COVID-19 which is the acronym of corona virus disease 2019. In the past 20 years additional coronavirus epidemic have occurred. SARS-CoV provoked a large scale epidemic is beginning in the China and involving two dozen countries with the 8000 case and the 800 deaths and the MERS-CoV that began in Saudi Arabia and has appximately 2500 cases and 800 deaths and still causes as sporadic cases. Thus new virus seems to be very contiguous and has quickly spread globally in a meeting January 30 2020 per the international health regulations (IHR2005) the outbreak was declared by WHO a public health emergency of international concern (PHEIC) as it had spread to 18 countries with 4 countries reporting humans-to-human transmission. and an additional landmark occurred on February 26 2020 as the first case if the disease

not imported from China was recorded in USA initially the new virus was called 2019-nCoV. Subsequently the task of experts of the international committee on the taxonomy of viruses (ICT) term it the SARS-CoV-2 virus as it is very similar to one that caused the SARS outbreak (SARS-CCoVs). The CoVs have become major pathogen of a emerging respiratory disease outbreak. They are a large family of sing stranded family of RNA viruses(+ssRNA) that can be isolated in different animal species. For reasons yet to be explained these viruses can cross species barrier and can cause in humans, illness ranging from the common cold to severe disease such as MERS and SARS. Interestingly these later viruses have probably originated from bats and then moving into other mammalian host the himalyan palm civet for SARS Cov-2 and the dromedary camel for MERS CoV before jumping to humans. The dynamics of SARS-CoV-2 are currently unknown but there speculation that it also has an animal origin. At the moment the therapeutic strategies to deal with the infection are only supportive and prevention aimed at reducing transmission in the community is our best weapon.

Plasma Therapy

Many options are being explored to treat Covid-19.These include new drugs specially designed to treat SARS-CoV-2 as well as repurposed drugs that is existing drugs designed to treat a different disease. By far the oldest treatment being tested, through is convalescent plasma. This involves the using of the blood plasma(BP) from people who have recovered from COVID-19 and infusing it into patients who currently have the disease.

Plasma is the liquid portion of blood that remains when all red and white blood cells have been removedi. It was over a 100 years ago that the Emil Behring was awarded the first Nobel prize for physiology and medicine for his work demonstrating that the plasma could be to treat diphtheria. We know that the key component of plasma for treating infection is antibodies. Antibodies are U-shaped Protein that are highly specific for whichever infection a person has previously encountered. They are produced in vast quantities by B-cells of our immune system in order to bind to the invading virus and target it for distruction. The concept of vaccination relies on stimulating antibody production to infection not yet met. In contrast, using the CP involves the transfer of antibodies form donor who have already mounted an immune response, thus offering immediate (But transient) protection to the recipient. Convalescent plasma has been trialled as a therapy in previous coronaviruses outbreak. There are few observational studies were that conducted during the first SARS epidemic in the 2003. These all are reported improvement in patients after receiving convalescent plasma, and no evidence of serious complication however, these studies were largely case reports not theost reliable type of evidence. Convalescent plasma treatment was also tasted during the Ebola virus outbreak in 2013-2016. Several case reports showed promising results, but again, larder – scale randomized trials were not performed. Still, the WHO published guidelines to appropriate use of plasma from recovered patients. The handful of early reports in which COVID-19 patients have been treated convalescent plasma have garnered plenty of interest. Each has concluded that plasma therapy is safe improves patients outcome, but there are significant limitations to each of these studies. To began with, each of study has only treated maximum 10 patients also, there where no control patients (people who weren't given convalescent plasma), so it's impossible to know how the patient may have responded without treatment. Fortunately, more extensive studies are now in the pipeline to provide robust evidence either for or against the use of convalescent plasma. World wide there are over 60 clinical trials actively recruiting covid-19 patients to study the effect of convalescent plasma. Patients are typically given about 500 ml plasma intravenously and their progress is then carefully monitored. Many studies are using plasma from non infected patient as the placebo arm of the trials, to ensure that any benefits indentifed are indeed specific for SARS-CoV-2 antibodies.

History

Coronavirus disease was first described in 1931 with the first coronavirus (HCoV-229E). Isolated from humans in 1965. Untill the outbreak of SARS in 2002 only two humans corona viruses (HCoV) were known-HCoV-229E and HCoV-oC43. Once the SARS coronavirus (SARS CoV) had been identified to further human corona viruses were identified. Three groups of corona viruses exist: Group 1(HCoV-229E and HCoV-NL63), Group 2 (HCoVOC43 and HCoV-HKU1) Group 3 (No human CoVs as yet). SARS-CoV is an outlayer to all there groups although Some places it in group 2.

Taxonomy

Coronaviruses and toroviruses are two genera within the virus family coronaviridae, order Nidovirales. Coronaviruses are well-established pathogen of human and animal while the toroviruses are recognized as causes of animal diarrhoea. Toroviruses have also been found in human faeces but their aetiological role remain unclear. Coronaviruses are classified into three groups initially based on antigenic relationship of the spike(S) membrane (M) and Nucleocapsid (N) Protein and now reinforced by viral genetic phylogeny (BOX 57.1). The HCoVs 229E and NL63 are group 1 corona virus while OC43, HKU-1 and SARS coronaviruses are classified in group 2. Group 3 coronaviruses are found in avian species. Genetic recombination readily occur between members if the same and of different coronaviruses groups providing opportunities for increased genetic diversity efforts yo identify the animal reservoir of SARS coronaviruses lead to the discovery of diverse bat coronaviruses in both groups 1 and 2 that are closely related phylogenetically to different mammalian coronaviruses it has been proposed that bat coronaviruses may indeed have been ancestors of many mammalian coronaviruses it is not worthy that recent studies on the comparative evaluation of animal and human coronaviruses have lead to the conclusion that HCoV229E and OC43, the causes of the common cold which are now globally endemic in humans crossed species from their

animal reservoirs (bats and cattles) to humans within the last 200 years illustrating the fact that coronaviruses continue to criss species barrier and cause novel disease.

TRANSMISSION

Person-to-Person Transmission

Experts believe on the virus that causes COVID-19 spreads mainly from the person to person. There are several ways this can happens. These are given as follows:-

Droplets

When an infected person may coughs, sneezes, or talks, droplets with the virus fly into the air from their nose or mouth. Anyone who is in the 6 feet of that person can breathe those droplets into the their lungs.

Airborne transmission

The Research shows that the virus can live in the air for up to 2-3 hours. When you breathe the air in to lungs that has the virus floating in it, it gets into your lungs. Surface transmission. Another way to catch the new covid 19 is when you touch the surfaces that someone who has the virus has coughed or sneezed on. You may touch the countertop or doorknob that's contaminated and then touch your nose, mouth, or eyes. The virus can live on the surfaces like a plastic and stainless steel for 2 to 3 days. To stop the contamination clean and disinfect all counters, knobs, and other surfaces you and your family touch several times a day. Fecal-oral. Studies also suggest that the virus particles can be found in the infected people's poop. But the experts aren't sure whether the infection can spread through contact with an infected person's stool. If that person is uses the bathroom and doesn't wash their hands, they could infect things and people that they touch. The corona virus most often spreads through the people who have symptoms. But it may be possible that to pass it on without showing any signs. Some of the people who don't know they've been infected can give it to others. This is called as asymptomatic spread. You also can pass it on before you notice any signs of infection, called presymptomatic spread.

Community Spread

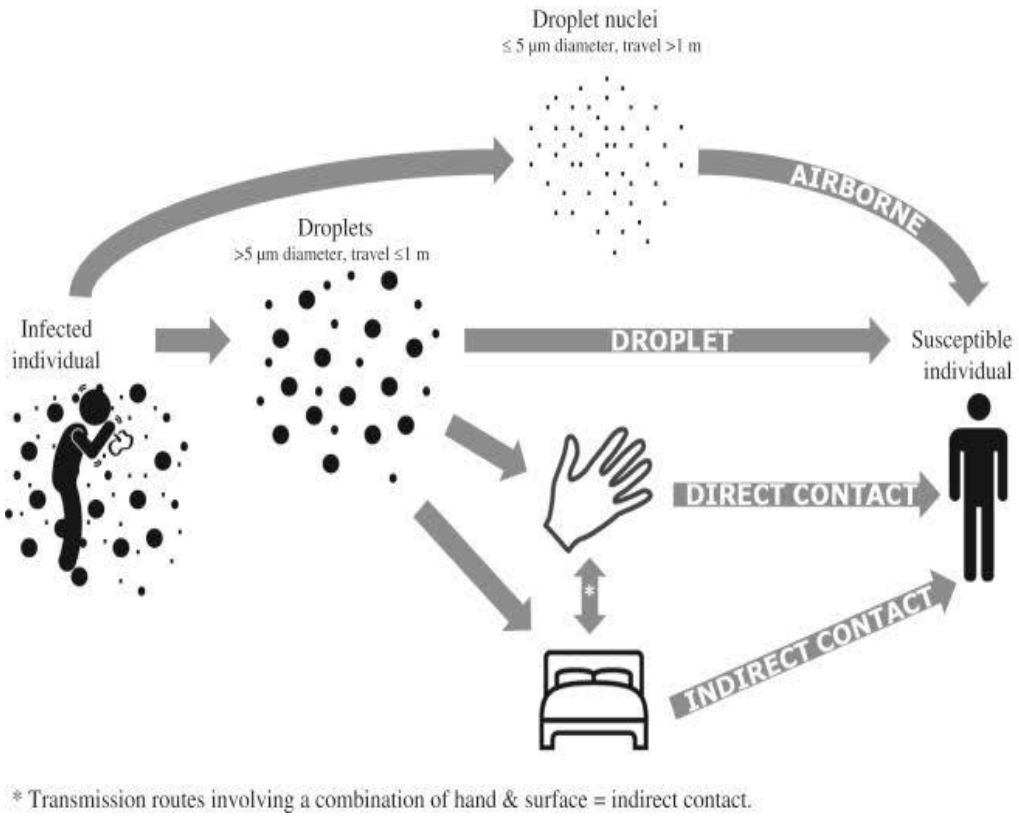
Sometimes, a person can trace how they got the virus because they know that they have been in contact with the someone who's sick. In other cases, the cause is complety unknown. The community spread is when someone gets the virus without any known contact with a sick person.

Pets and COVID-19

A few pets have tested positive for the new virus. Not all of these animals are had signs of illness, but some have had mild symptoms. The animals may have caught the coronavirus from the close contact with humans who were infected. Public health officials say that they are still studying COVID-19 but that there's no evidence that pets play a role in its spread.

How Easy Is It to Get Infected

Researchers say that on a average, every person who has the COVID-19 will pass it on to 2 or 2.5 others. One study says that the number is even higher, with one sick person infecting between 4.7 and 6.6 others. By the comparison, someone who has the flu will.



(Fig. Transmission of Corona Virus)

Probably give it to an average of 1.1 to 2.3 others. But anyone person with measles might spread it to 12 to 18 others.

Can I Get Infected From Delivery Food, Packages, or Groceries?

It is the highly unlikely that you'll catch COVID-19 from packages, groceries, or food. The important thing is that to limit your contact with other people. If you do your own shopping. Then try to keep at least 6 feet away from others in the store. That may not be possible all the time, so wears cloth mask, too. If you are use delivery service, have them leave groceries, food, or packages outside your front door if you can.

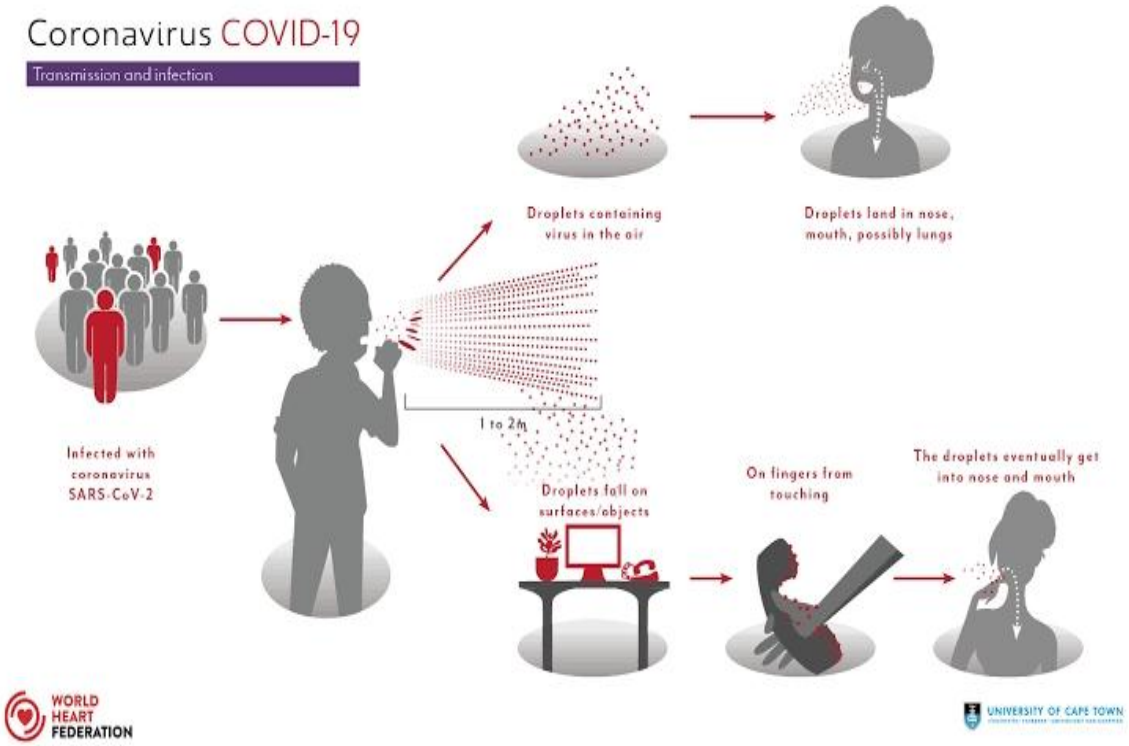


Fig. Spread of Covid 19.

TREATMENT

Currently, there is no antiviral medication is recommended to treat COVID-19 and no cure is available for COMID-19. Antibiotics are not effective against viral infections such as COvID-19. The On going research is studying various drugs that may be effective for the treating severe COVID-19. Treatment is directed at relieving symptoms and may include:

Pain relievers (ibuprofen or acetaminophen) Cough syrup or medication Rest Fluid intake. There is no evidence that ibuprofen or other nonsteroidal anti-inflammatory drugs (NSAIDS) need to be avoided. If you have mild symptoms, your doctor may recommend that you recover at home. He or she may give you the special instructions to monitor your symptoms and to avoid the spreading the illness to others. You may be asked to isolate yourself as much as possible from family and pets while you're sick, wear a mask when you're around people, and to use a separate bedroom and bathroom. Your doctor will likely recommend that you stay in home isolation for a period of time except to get medical care. Doctor will be likely follow up with you regularly. Check with your doctor about that when you can end home the isolation. If you are very ill, then you may need to be treated in the hospital.

PLASMA THERAPY

Researchers are testing the use of donated blood as a treatment for people with severe coronavirus disease 2019 (COVID-19). People who've recovered from Covid-19 have antibodies to the disease in their blood. Doctors call this convalescent plasma. The Researchers hope that the convalescent plasma can be given to the people with severe COVID-19 to boost their ability to fight the virus. The U.S. Food and Drug Administration has outlined the requirements that individuals must meet to donate blood for this research. Before donated blood can be used, it must be tested for safety. It then goes through a process to separate out blood cells so that all that's left is plasma with antibodies. A immediate goal of this research is to determine if the convalescent plasma can improve the chance of recovery for people with the most severe disease. A second goal is to test whether convalescent plasma can help keep people who are moderately sick from getting sicker. Such a treatment would be a boon for people athigh risk- such as with underlying medical conditions, as well as

family members and health care workers who have been exposed. In addition, learning more about the use of convalescent plasma now will help health care workers be better prepared if a second wave of disease occurs, as has happened with past viral outbreaks. To find out if you may be eligible for this treatment, talk with your doctor. If you've had and recovered from Covid-19, consider donating blood through the American Red Cross or your local donation center. They can provide information about the donation process.

Drugs which Might used in Treatment of COVID 19

The World Heath Organization (WHO) announced that they have helped to launch four "mega trials" against COVID-19 and there are numbers of more smaller ones Coordinated in nations worldwide. The WHO-backed trials are mainly focusing on drugs that are prevent coronavirus that thought to directly block SARS-CoV-2 the virus strain that causes COVID-19 – from replicating inside humans lungs. Below are the some of the main drugs these trials are looking at.

Remdesivir

The Remdesivir is an intravenous administer antiviral drug that was developed to block infection that is related coronaviruses and even also Ebola virus, and is one of the drugs the WHO is helping to investigate. Remdesivir has already shown to action against SARS-CoV-2 in cells in a dish in a lab as well as also in mice infected with the virus. Remdesivir is specifically targets the key viral proteins involved in making new copies or duplicate of the virus and prevents them from their working. Remdesivir has already been used in some COVID-19 patients in the United States and appears good results and safe administration, but more trials are needed to really know it's proper action on human body in this case.

Lopinavir/ritonavir

This is a drug mainly used in combination form against viruses like HIV. It's action or work in a similar way to Remdesivir by blocking key viral proteins this is mainly called "proteases". Lopinavir/ritonavir also been shown to be effective against SARS-CoV-2 in lab cells as well as in mice same as Remdesivir and also is being tested alongside anantiviral drug called "interferon beta". This is currently in medical field used to treat Multiple sclerosis and can stimulate or enhance the natural defences of the body's cells against COVID-19.

Chloroquine and hydroxychloroquine

These both of the drugs are currently used to cure or treat malaria disease and also the autoimmune disease lupus. The Chloroquine has been studying against the lots of different infections diseases because in the lab it can block viruses - including SARS-CoV-2 - from getting inside cells placed in a dish and so prevent infection. At outside the lab, chloroquine has not been demonstrated to have a profound action at preventing disease and there is an limited evidence available that it can work for COVID-19, despite receiving a lot of hype from US President Donald Trump. But again, it required large number of trials and the WHO is supporting these. Precautions should be observed with chloroquine as it can have significant side effects with a certain adverse effect in a certain people and may even these block the immune response – the desired action in lupus treatment.

Two other options

The above potential treatments all are mainly work by blocking some key element of the virus infection machinery using small molecules. There is also two other kinds of treatments are also being explored in trials that work in a different way. The first treatment is passive immunisation which is the transfer - or transfusion – of potential protective antibodies form someone who has been infected all ready and recovered from COVID-19 to the patient who is at high-risk or is suffering from a SARS-CoV-2 infection. This is called "Convalescent Sera" (which is a purified blood product from Someone who has recovered from COVID-19) can block SARS-CoV-2 in cells in a dish in the lab and has the required potential to help develop treatments. Passive immunisation for COVID-19 is being tested in number of trials across the world and so far results seem to suggest it is an safe treatment to use. Another kind of possible treatment works by blocking parts of suffering patient own immune systen that are likely overreacting to SARS-CoV-2 infection and contributing to the damage in our lungs. In the limited studies that have been conducted on COVID-19, it as been seens that in Some severe cases our immune response goes into overdrive without being able to clear the infection and this can lead to increase the severity of the disease. When this happens, the high levels of inflammation is found at the lungs. Potential treatments that is look at blocking the immune components linked to this kind of severity have begun. That's why, extreme caution must be taken when the manipulating the immune response during an infection as in the absence of other therapies we mainly available on our immune response to limit the virus replicating. So although there is not specific treatments for COVID-19 are yet available, drugs are being tested and clinical trials and starting to yield results. This, combined mainly with the further knowledge that scientists are gaining about SARS-CoV-2 will help massively to found the vaccine for Covid-19.

Prevention

Clean your hand often. use soap and water, or an alcohol based hand rub. Maintain a safe distance from anyone who is coughing or sneezing.

Don't touch your eyes, nose or mouth.

Covr your nose and mouth with your bent elbow or a tissue when you cough or sneeze.

Stay home if you feel unwell

If you have a fever, cough and difficulty in breathing, seek medical attention. Call in advance

Follow the directions given by your local health authorities.

CONCLUSION

• This new virus outbreak has challenged the economic, medical and public health infrastructure of China and to some extent, of other countries especially, it's neighbours.

Time alone will tell how the virus will impact our lives here in india more so, future outbreak of viruses and pathogens of zoonotic origin and likely to continue. Therefore, apart from curbing this outbreak, efforts should be made to devise comprehensive measure to prevent future outbreaks of zoonotic origin. There are hundreds of Corona viruses, most of which circulate in animals only seven of these viruses infect humans and four of them cause symptoms of common cold. But, three times in the last 20 years, a coronavirus has jumped from animal to human to cause several disease. SARSSARS, a beta coronavirus emerged in 2002 and was controlled mainly by aggressive public health measures. There have been no new cases since 2004. MERS emerged in 2012, still exists in camels, and can infect people who have close contact with them. COVID-19, a new and sometimes deadly respiratory illness that is believe to have originated in a live animal market in China, has spread rapidly throughout that country and the world. The new coronavirus was first detected in wuhan, China in December 2019. Tens of thousands of people were infected to China, with the virus spreading easily from person to person in many parts of that country. The novel coronavirus infection were at first associated with travel from wuhan, but the virus has now established itself in 177 countries and territories around the world in a rapidly expanding pandemic. Health official in the United States and around the world are working to contain the spread of the virus through public health measures such as social distancing, contact tracing, testing, quarantine and travel restrictions to treat the disease and to develop a vaccine. The World Health Organization declared the novel coronavirus outbreak a public health emergency of international concern on January 30. On March 11,2020 after sustain spread of disease outside to China, the World Health Organization declared the COVID-19 epidemic a pandemic. Public health measures like once implemented in China and now around the world, will hopefully blunt the spread of the virus while treatments and a vaccine are developed to stop it.

REFERENCESS

1. Brainin M. Stroke care and COVID19 pandemic words from our President, www.worldstroke.org/news-and-blog/news/stroke-care-and-the-covid19-pandemic (11 April 2020).
2. Henry BM, Aggarwal G and Lippi G .Cerebrovascular disease is associated with an increased disease severity in patients with coronavirus disease 2019 (COVID-19): a pooled analysis of published literature. Int J Stoke. Epub ahead of print 20 April 2020.
3. Bersano A and Pantoni L. Being a neurologist in Italy at the time of the COVID-19 outbreak. Neurology. 3 April 2020.
4. Rothwell, Coull, AJ, Lovett, JK, PM. The Oxford Vascular Study Clinical guide for the management of stroke patients during the coronavirus pandemic 23 March 2020 Version 1, www.england.nhs.uk/coronavirus/wp-content/uploads/sites/52/2020/03/C033Specialty-guide_-Stroke-and-coronavirus-v1-24March_.pdf (11 april 2020).
5. McAuley, DF, Mehta P, Brown, M, Sanchez, E, Tattersall, RS, Manson, JJ. HLH Across Speciality Collaboration UK. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet. Epub ahead of print 2020.
6. Akhmerov, A, Marban, E. COVID-19 and the heart. Circ Res. 7 April 2020.
7. Stone M, Poyiadji, N, Shahin, G, Noujaim, D, Patel, S, Griffith, B. Coronavirus associated acute hemorrhagic necrotizing encephalopathy: CT and MRI features. Radiology. 31 March 2020.
8. Feigin V, Béjot, Y, Reis, J, Giroud, M. A review of the epidemiological research on stroke and dementia and exposure to air pollution. Int J Stroke, 2018; 13: 687–695.
9. The Management of acute ischemic stroke in patients with COVID-19 infection: report of an international panel. Int J Stoke 2020.
10. Klein BC, Busis, NA. COVID-19 is catalyzing the adoption of teleneurology. Neurology.1 April 2020.
11. Temporary emergency guidance to US stroke centers during the COVID-19 pandemic. Stroke.1 April 2020.
12. Markus HS, Walsh, J. Telemedicine for follow-up of rare neurological disease. Stroke, 2019; 50: 750–753.
13. Yancy CW. COVID-19 and African Americans. JAMA. 15 April 2020.
14. The COVID-19 host genetics initiative, www.covid19hg.org (17 April 2020).
15. Lai, M.M. The SARS virus: the beginning of the unraveling of a new coronavirus. J.
16. Drosten, C. et al. ldentification of a COVID 19 in patients with severe acute.
17. Martina, B.E. et al. Virology: The SARS virus infection of cats and ferrets. Nature 425.
18. Guan, Y. et al. Isolation and The characterization of viruses related to the SARS.
19. Marra, M.A. et al. The genome sequence of the SARS-associated coronavirus. Biomed. Sci., 2003; 10: 664-675. The respiratory syndrome. N. Engl. J. Med., 2003; 348: 1967-1976. 915 (2003).
20. The virus from animals in southern China. Science, 2003; 302: 276-278. Science, 2003; 300: 1399 -1404.
21. Snijder, E.J. et al. Unique and conserved features of genome and the proteome of SARS-coronavirus, an early split-off from the covid 19 group 2 lineage. J. Mol. Biol., 2003; 331: 991-1004.
22. Hamparian, V.V. Rhinoviruses. In the Diagnostic Procedures for Viral, The Rickettsial and Chlamydial Infection. (eds. Lennette, E.H. & Schmidt, N.J.) 562 (American Public Health Association, Washington DC, 1979).
23. Rota, P.A. et al. Characterization of a COVID 19 associated with severe acute respiratorysyndrome. Science, 2003; 300: 1394-1399.
24. Zhang CT, Chen, L.L., Ou, H.Y., Zhang, R. & ZCURVE_CoV: The new system to recognizeprotein coding genes in coronavirus genomes, and its applications in analyzing SARS-CoVgenomes. Biochem. Biophys. Res. Commun, 2003; 307: 382-388.

25. Sawicki, S.G. & Sawicki, D.L. The discontinuous extension of COVID 19 use for synthesis ofsubgenome-length negative strands. Adv. Exp. Med. Biol., 1995; 380: 499-506.
26. van Marle, G. et al. The Arterivirus discontinuous mRNA transcription is guided by basepairing between sense and antisense transcription-regulating sequences. Proc. Natl. Acad. Sci. USA, 1999; 96: 12056-12061.
27. Lole, K.S. et al. The human immunodeficiency virus type 1 genomes from subtype C-infected seroconverters in India, with evidence of intersubtype recombination. J. Virol., 1999; 73: 152-160.
28. The two open reading frames located downstream of the S gene of bovine coronavirusby Mounir S and Talbot PJ.
29. Duarte, M. et al. Sequence analysis of the porcine epidemic diarrhea virus genome befween the nucleocapsid and spike protein genes reveals a polymorphic ORF. Virol., 1994; 198: 466-476.
30. Godet, M., Grosclaude, J., Delmas, B. & Laude, H. A neutralization determinants and themajor receptor binding are located within the same domain of the transmissible gastroenteritis virus (coronavirus) spike protein. J. Virol., 1994; 68: 8008-8016.
31. Zelus, B.D., Bonavia A, Wentworth, D.E., Talbot, P.J. & Holmes K.V. The Identification of areceptor-binding domain of the spike glycoprotein of human coronavirus HCoV-229E. J. Virol., 2003; 77: 2530-2538.
32. Ksiazek, T.G. et al. A novel COVID 19 associated with severe acute respiratory syndrome. N. Engl. J. Med., 2003; 348: 1953-1966.
33. Vaughn, E.M., Halbur, P.G. & Paul, P.S. Sequence comparison of the porcine respiratory coronavirus isolates reveals heterogeneity in the S, 3, and 3-1 genes. J. Virol.
34. Mclntosh, K. et al. Coronavirus infection in acute lower respiratory tract disease.
35. PenePene, F. et al. Coronavirus 229E-related pneumonia in iimmunocompromise.
36. Boivin, G. et al. Human metapneumovirus infections in hospitalized children, 1995; 69: 3176-3184. infants. J. Infect. Dis., 1974; 130: 502-507. patients. Clin. Infect. Dis., 2003; 37: 929-932. Emerg. Infect. Dis., 2003; 9: 634-640.
37. Myint, S., Johnston, S., Sanderson, G. & Simpson, H. Evaluation of the nested polymerase chain methods for the detection of human coronaviruses 229E and OC43.Mol. Cell. Probes, 1994; 8: 357-364.
38. Stephensen, C.B., Casebolt, D.B. & Gangopadhyay, N.N. Phylogenetic analysis of the highly conserved region of the polymerase gene from 11 coronaviruses and development of a consensus polymerase chain reaction assay. Virus Res., 1999; 60: 181-189.
39. Boom, R. et al. A rapid and the simple method for purification of nucleic acids. J. Clin. Microbiol, 1990; 28: 495-503.