Asian Journal of Immunology

3(4): 15-29, 2020; Article no.AJI.56272

Structure and Function of COVID-19 Encode Proteins in the Transcription and Replication Mechanism with Its Preventive Measures and Propose Efficacy Treatments: A Critical Systematic Review

Oguh C. E.[1*], Obiwulu E. N. O.[2], Oniwon W. O.[3], Okekeaji U.[4], Ugwu C. V.[1], Umezinwa O. J.[5] and Osuji C. A.[1]

[1]Department of Biochemistry, University of Nigeria, Nsukka, Enugu State, Nigeria.
[2]Department of Integrated Science, Delta State College of Education, Agbor, Delta State, Nigeria.
[3]Department of Biochemistry, Kogi State University, Anyigba, Nigeria.
[4]Department of Pharmaceutical Microbiology and Biotechnology, University of Nigeria, Nsukka, Enugu State, Nigeria.
[5]Department of Science Laboratory Technology, University of Nigeria, Nsukka, Enugu State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author OCE designed the study, wrote the protocol and manage the analyses of the study. Author OENO wrote the first draft of the manuscript. Authors OWO, OU, UCV and UOJ managed the literature searches. Author OCA read and edit the final manuscript. All authors read and approved the final manuscript.

Article Information

Editor(s):
(1) Dr. Cynthia Aracely Alvizo Báez, Autonomous University of Nuevo Leon, Mexico.
Reviewers:
(1) Loc Nguyen, Vietnam.
(2) Lívia Garcia Bertolacci-Rocha, Universidade Federal de Goiás, Brasil.
(3) Mujeeb Shittu, Michigan Technological University, USA.
Complete Peer review History: http://www.sdiarticle4.com/review-history/56272

Review Article

Received 03 April 2020
Accepted 25 April 2020
Published 02 May 2020

ABSTRACT

The sudden occurrence outbreak of coronavirus disease in 2019 (COVID-19) by the severe acute respiratory syndrome coronavirus2 (SARS-CoV-2) poses a serious harm worldwide and local economies. Due to high numbers of infection and death, the pandemic calls for an urgent demand of active, effective, affordable and available drugs to control and diminish the pandemic. Coronavirus disease 2019 is a public health unexpected and sudden crisis which required action of

**Corresponding author: E-mail: collinsoguh@gmail.com;*

international concern. At present there is no generally recognized effective pharmaceutical treatment to the disease, although it is to a great extent for patient contracting the severe form of the disease. The development of new strategies to prevent or control the spread of COVID-19 infections and the understanding of the virus replication, and pathogenesis required immediate action. Therefore, this systematic review was to investigate the biochemical effect of the virus in human, symptoms, prevention, statistics cases and summarize the evidence regarding chloroquine and hydroxychloroquine for the treatment of COVID-19.

Keywords: Coronavirus; mechanism; prevention; structure; treatment.

ABBREVIATIONS

COVID-19: Coronavirus disease 2019; SARS-CoV2: Severe acute respiratory syndrome coronavirus 2; S: Spike; M: Membrane; E: Envelope glycoproteins; HE: Hemagglutinin Esterase: N: Nucleocapsid protein; RdRp: RNA-dependent RNA polymerase; MHV: Mouse hepatitis virus; ACE2: Angiotensin-converting enzyme 2; 3CLpro: Coronavirus main protease; PLpro: Papain-like protease; FECV: Feline Enteric Coronavirus; FIPV: Feline Infectious Peritonitis Virus; TGEV: Transmissible Gastro-Enteritis Virus; PEDV: Porcine Epidemic Diarrhea Virus; PRCoV: Porcine Respiratory Coronavirus; CCoV: Canine coronavirus; BCoV: Bovine Coronavirus; gRNA: RNA genome; DMV: Double-Membrane Vesicles; RTC: Replication and Transcription Complex; nsp3: non-structural protein3; CQ: Chloroquine; HCQ: Hydroxy-chloroquine; EEs: Early endosomes; ELs: endo-lysosomes; Cryo - EM: Cryo electron microscopy; IFA: Immune fluorescence analysis; ERGIC: Endoplasmic reticulum-Golgi apparatus intermediate compartment.

1. INTRODUCTION

Coronavirus is a novel disease that had not been previously identified in human of recent. Coronavirus disease 2019 is a respiratory disease illness that is transitive from one individual to the other. The type of virus that causes COVID-19 is a new inventive disease that was firstly recognize during a survey outbreak in December. 2019 at Wuhan, China, this disease has spread to more than 213 countries with 2,931,787 confirmed cases and over 203,596 confirmed deaths worldwide as of April 26, 2020 [1] with U.S, Spain, Italy and France as the most affected countries with 960,896, 223,759, 195,351 and 161,488 confirm cases respectively. The name coronavirus, coined in 1968, is derived from the corona-like or crown-like morphology observed for these viruses in the electron microscope. The virus is highly contagious and thousands of new cases are reported around the world every day. The risk of infection from the virus that causes COVID-19 is higher for people who have close contacts with people with the disease, such as healthcare workers and household members and other people at higher risk for this infection are those who are living in an area with ongoing spread of COVID-19 or those who traveled to effected place. Risk of death is only higher in older people (above an age of 60 years) and people with pre-existing health conditions. Coronaviruses infect both animals and humans, especially animals such as bats, this animal host the largest variety of corona viruses shows to be immune to corona virus-induced illness in human [2]. Researchers have been working to trace the achievable treatments in order to save lives and produce vaccines for future prevention and control. The viruses infect a variety of organisms such as human and animal host cells, and also carry out their infection and replication on the host. Also, many proteins located on the virus have most important function in the replication mechanism, although the role is yet to be defined. In this case, it is important to investigate the role and importance of these proteins during mechanism. Therefore; this review explains the structure, classification, symptoms, prevention, statistics cases and summarize the evidence regarding chloroquine and hydroxychloroquine for the treatment of COVID-19.

1.1 Structure of Covid-19

Coronaviruses are enveloped viruses with round and sometimes pleiomorphic virions of approximately 80 to 120 nm in diameter (Fig. 1). The coronaviruses also contain positive-strand RNA, which have the largest RNA genome (approximately 30 kb) reported to date (178, 196). Corona viruses (COVID-19) are mainly large viruses containing a single-stranded positive-sense RNA genome protected by membrane within a membrane envelope within the virus. The viral membrane is covered with

glycoprotein spikes that give coronaviruses their crown like appearance. COVID-19 virus encode five structural proteins in their genomes. These are the Spike (S), Membrane (M), Envelope (E) glycoproteins, Hemagglutinin Esterase HE and Nucleocapsid N protein, (Fig. 1). The virus envelope proteins and N protein is embedded in all virion but HE is only present in some beta coronaviruses. In addition to that, it is thought the virus particles are huddled together owing to interaction between these proteins [3,4].

1. **S Glycoproteins:** They are located outside the virion which gives the virion the typical shape. The S proteins form homotrimers, which allow the formation of sun-like morphologies that give the name of Coronaviruses [5]. S proteins bind to the virion membrane via the C-terminal transmembrane regions, also they interact with M proteins [6]. The Virions is attach to a specific surface receptors in the plasma membrane of the host cell through the N-terminus of the S proteins. This S glycoprotein initiate host cell invasion by both SARS-CoV and SARS-CoV-2 via binding to a receptor protein called angiotensin-converting enzyme 2 (ACE2) which is located on the surface membrane of host cells [7,8]. A study conducted by [9, 10], shows that the invasion process requires S protein priming which is accompany by the host cell produced serine protease. Also the viral genome also encodes several nonstructural proteins which include the RNA-dependent RNA polymerase (RdRp), coronavirus main protease (3CLpro) and papain-like protease (PLpro) [9,10].
2. **M Glycoproteins:** The M Glycoproteins on the virus have three transmembrane regions. The proteins are glycosylated in the organelle called Golgi apparatus [11]. This reformation of the M protein is important for the virion to fuse into the cell and to make protein antigenic. The M protein plays a key important role in stimulate virions in the cell. The virus N protein also forms a complex by binding to genomic RNA and the M protein elicit the production and formation of interacting virions in this endoplasmic reticulum-Golgi apparatus intermediate compartment (ERGIC) with this complex [12,13].
3. **E Glycoproteins:** This are small proteins that are constitute of just about 76 to 109 different amino acids. Approximately 30 different amino acids in the N-terminus of the E proteins permit attachment to the membrane of viruses [14]. In addition, coronavirus E proteins play a critical important role in pulling together and morphogenesis of virions within the cell. A study of coronavirus E and M proteins were expressed together with mammalian expression vectors to form virus-like structures within the cell [15]. In a study, there was a great decrease in the ability of the recombinant mouse hepatitis virus (MHV) and SARS viruses to elicit E protein expression in the genome to support this status [16,17].
4. **N Proteins:** They are phosphor proteins that are capable of binding to helix and have elastic structure of viral genomic RNA. It plays a crucial role in virion structure, replication and transcription of coronaviruses, because the N protein focus in both the replication and the transcriptional region of the coronaviruses and the ERGIC area where the virus is collected [16,17]. (Fig. 2).

1.2 Classification of Coronaviruses

The classification of Coronaviruses is based on genomic organization, resemblance in genomic sequence, antigenic properties of viral proteins, replication strategies and structural characteristics of virions, pathogenic, cytopathogenic and physicochemical properties [18]. The Coronaviruses are species of virus belonging to the *Nidovirales* order, which comprises *Coronaviridae, Arteriviridae, Roniviridae and Mesoniviridae* families [19]. The *Arteviridae* family consist of swine and equine pathogens, and the *Roniviridae* family is composed of invertebrate viruses. The *Coronaviridae* family is the largest among the four families, range from 26 to 32 kb by its genomic sizes of *coronaviridae* [20]. *Coronaviridae* virus family subdivided into two subfamilies, *coronavirinae* and *torovirinae*. *Coronavirinae*is gulf into four genera, Alpha coronavirus, Beta coronavirus, Gamma coronavirus and Delta coronavirus (Fig. 3). Alpha coronaviruses type 1 species are classified into the porcine TGEV (Transmissible Gastro-Enteritis Virus), feline FCoV, FECV (Feline Enteric Coronavirus) and FIPV (Feline Infectious Peritonitis Virus), Porcine PEDV (Epidemic Diarrhea Virus), PRCoV (Porcine Respiratory Coronavirus) and the canine CCoV. Alpha coronaviruses also agree with human CoVs such

as HCoV-229E and HCoVNL63, but various bat Coronaviruses.

Beta coronaviruses infect a wide varieties of mammalians, with species such as human with SARS-CoV, mice, HCoV-OC43, HCoV-HKU1, and MERS-CoV, Bovine Coronavirus (BCoV), and Murine coronavirus (MHV). Likewise to SARS-CoV and MERS-CoV, SARS-CoV-2 violence the lower respiratory system to cause viral pneumonia in human, and may also affect the heart, kidney, gastrointestinal system, liver, and central nervous system leading to multiple organ failure [21,22]. Current information indicates that SARSCoV-2 is more transmissible and very contagious than SARS-CoV [23].The beta coronavirus genome encrypt more than a few or several structural proteins, including the glycosylated spike (S) protein that functions as a major inducer of host immune responses. Gamma coronaviruses are exact of birds, with one exclusion of a beluga whale Coronavirus. The delta coronavirus genus was generated in 2012 and with various groups (HKU11, HKU12, HKU13) Coronavirus from mammals to birds [24] Fig. 3.

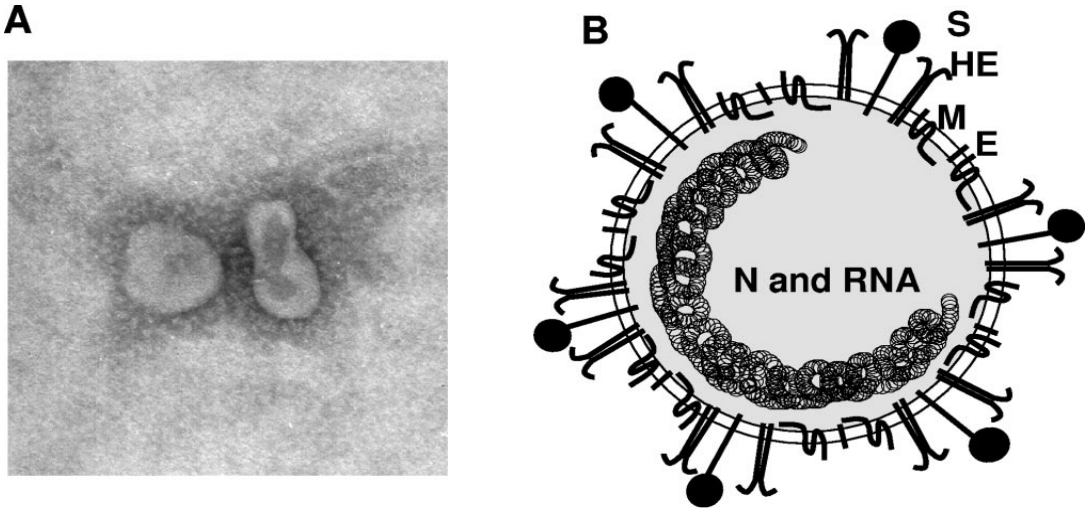

Fig. 1. Coronavirus virion. (A) Electron micrograph of mouse hepatitis virus MHV particles. (B) Schematic of virion

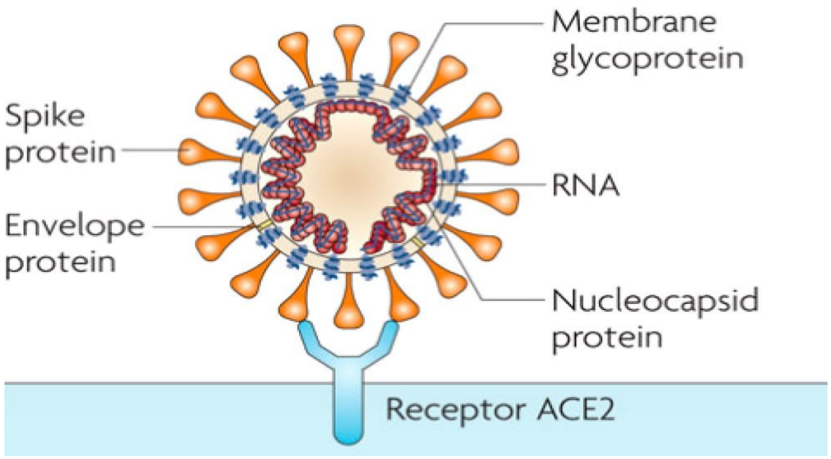

Fig. 2. Cartoon illustration of the coronavirus structure and viral receptor ACE2 on the host cell surface

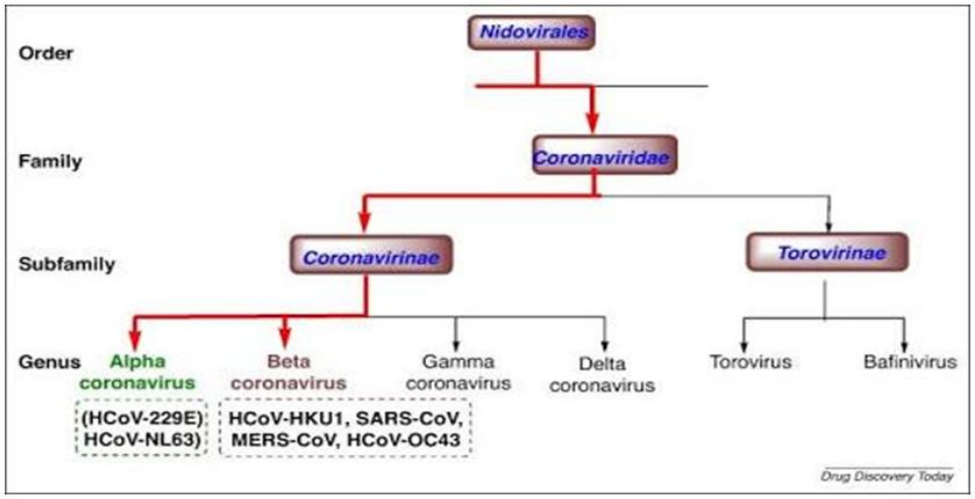

Fig. 3. Classification of Covid-19

2. COVID-19 MECHANISM OF ACTION (REPLICATION AND TRANSCRIPTION)

Coronavirus entry starts with the S protein binding to a target receptor on the cell surface, where after fusion is mediated at the cell membrane, delivering the viral nucleocapsid inside the cell for subsequent replication. The action of coronaviruses replication occurs in the cytoplasm of the host cell. The viruses mainly bind to the host receptor cell surface through the spike (S) protein. A conformational structure occurs in the structure and the process of entry into the virus cell start when S protein is bound to the receptor [25]. This process with endocytosis is reliant of pH through the receptor. The virus particle releases the RNA genome after going into the cytoplasm. This genome is a non-segmented RNA virus with the largest known RNA genome (gRNA), single-stranded, which is approximately 26-32 kb. The genome consists of seven several genes. It is prearranged into 5' non-structural protein coding regions include the replicase genes (gene 1), which are two-thirds of the genome, and 3' structural and nonessential accessory protein coding regions including the gene 2-7 [26]. The replicase gene 1 products are encoded two very large open reading frames ORF1a and 1b, which are interpreted or translated into two large polypeptides pp1a and pp1b, which are produce and synthesized directly from the 5' two-thirds of the genomic RNA of CoV (Fig. 4).

After the protein have been synthesized, which is made up of 16 units, non-structural protein (nsp1 to nsp16) is change with the influence of viral proteases pp1a and pp1b [27]. Same time, the Double-Membrane Vesicles (DMV) is virus Replication and Transcription Complex (RTC) [28,29]. The 16 unit's proteins form DMV. These nsp proteins, particularly non-structural protein3 (nsp3), have an important function in the virion structure, and the replication and transcription of CoV [30,31]. Genes 2 to 7 are transform from sub genomic mRNA. Sub genomics RNAs encode the main viral Envelope protein (E), structural proteins (S), Membrane protein (M), Nucleocapsid protein (N), and the accessory proteins, which are vital for virus-cell receptor binding. The recently structural synthesized proteins are released into the endoplasmic reticulum. All of these proteins, along with the N protein, are connected to the viral genomic RNA and localized in the ERGIC region [28,32] as shown in Fig. 4.

Although, N protein is known to be very essential for coronavirus replication, the exact function or role that this protein plays in this process remains unidentified. But, several research suggested that N protein interact with nsp3, which plays a critical and important role in the virus replication early in infection. The communication between viral S protein and Angiotensin-converting enzyme 2 (ACE2) on the host cell surface is of important interest since it initiates the infection process. Cryoelectron microscopy (Cryo-EM) structure analysis has make known that the binding affinity of SARS-CoV-2 S protein to ACE2 is approximately 10−20 times higher than that of SARS-CoV S protein binding to ACE2 [33,34]. It is consider that the binding affinity may contribute to the reported

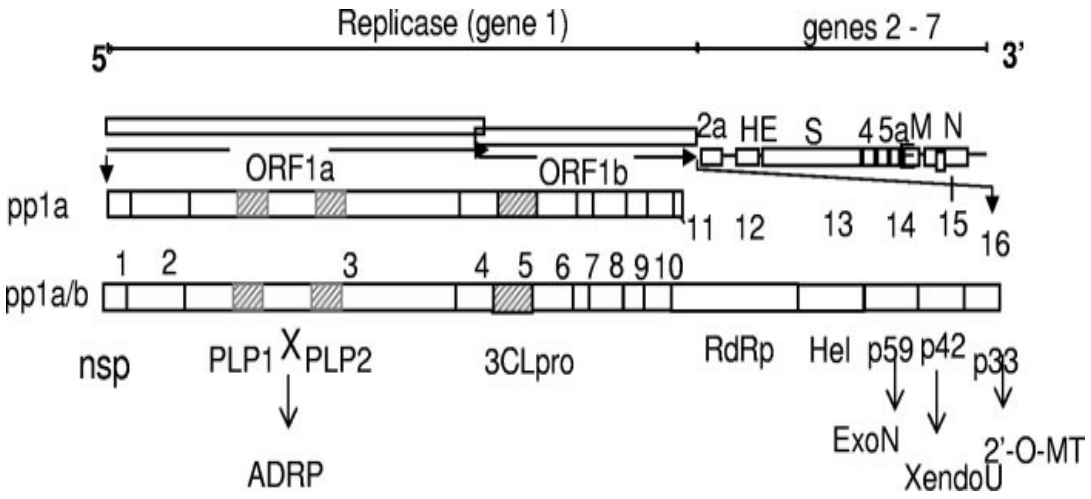

Fig. 4. MHV genome organization and replicase proteins

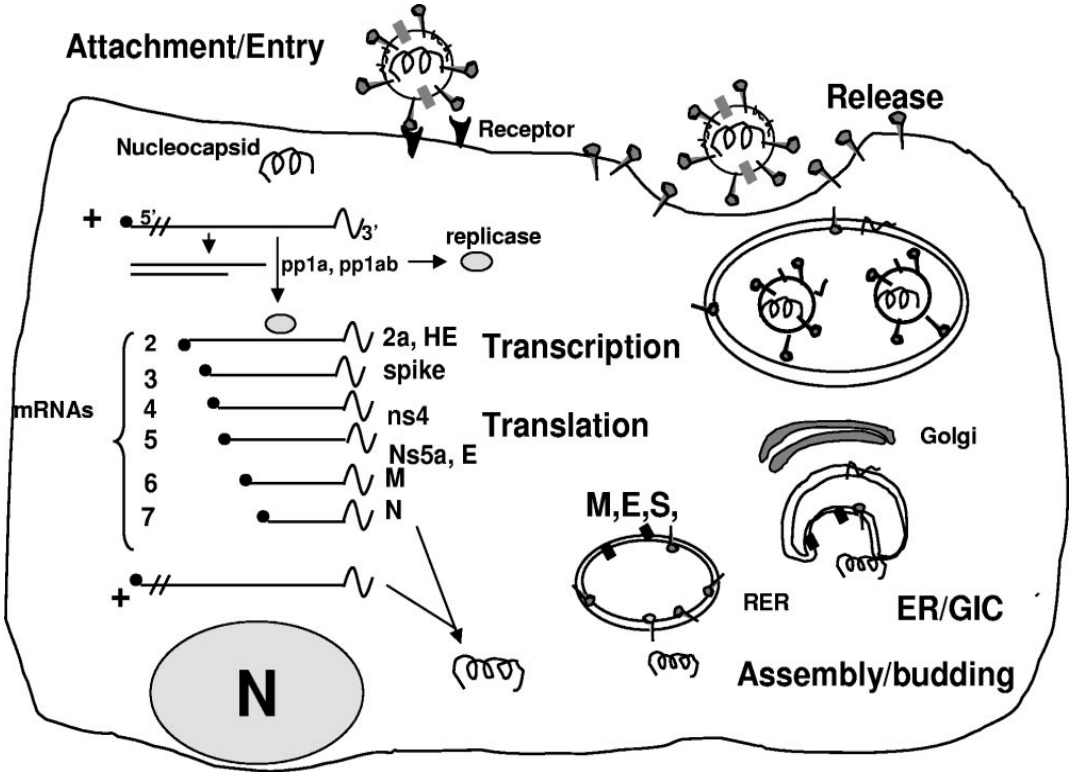

Fig. 5. Life cycle of coronavirus in a host cell

higher transmissibility spread and contagiousness of SARS-CoV-2 as compared to SARS-CoV [23].

The search also exists for discovery a breakthrough of therapeutic agents such as drugs and vaccine for targeting the highly preserved proteins related with both SARS-CoV and SARS-CoV-2 proteins [34-37]. RNA-dependent RNA polymerase (RdRp) and Coronavirus main protease (3CLpro) of SARS-CoV-2 share over 95 % of sequence similarity with those of SARS-CoV notwithstanding the fact that these two viruses shows or demonstrate only 79 % sequence similarity at the genome level proteins [34-37]. On the foundation of

sequence arrangement and homology modeling, SARS-CoV and SARS-CoV-2 share an extremely preserved receptor-binding domain (RBD), a domain of S protein and 76% of sequence similarity in their S proteins [34-37]. Although the Papain-like protease (PLpro) sequences of SARS-CoV-2 and SARSCoV are only 83% similar, they share the same active sites [35].

3. TRANSMISSION OF COVIDS-19

The virus that causes COVID-19 most likely emerged or come out from an animal source, which is now spreading from one person to the other. Via respiratory droplets generate when an infected individual sneezes or coughs can extend the spread of the virus to close contact (within about 6 feet length). The COVID-19 can also be contracted by touching a surface or object that has been contaminated with the virus from an infected person and then touching their own mouth, nose, or perhaps their eyes.

3.1 Symptoms of Covid-19

Patients with COVID-19 will eventually have slight to severe respiratory illness with symptoms of:

- Fever
- Cough
- Running nose
- Sore throat
- Body Ache
- Shortness or difficulty breathing
- At severe complications from this virus such as pneumonia in the lungs, multi-organ failure and in some cases lead to death.

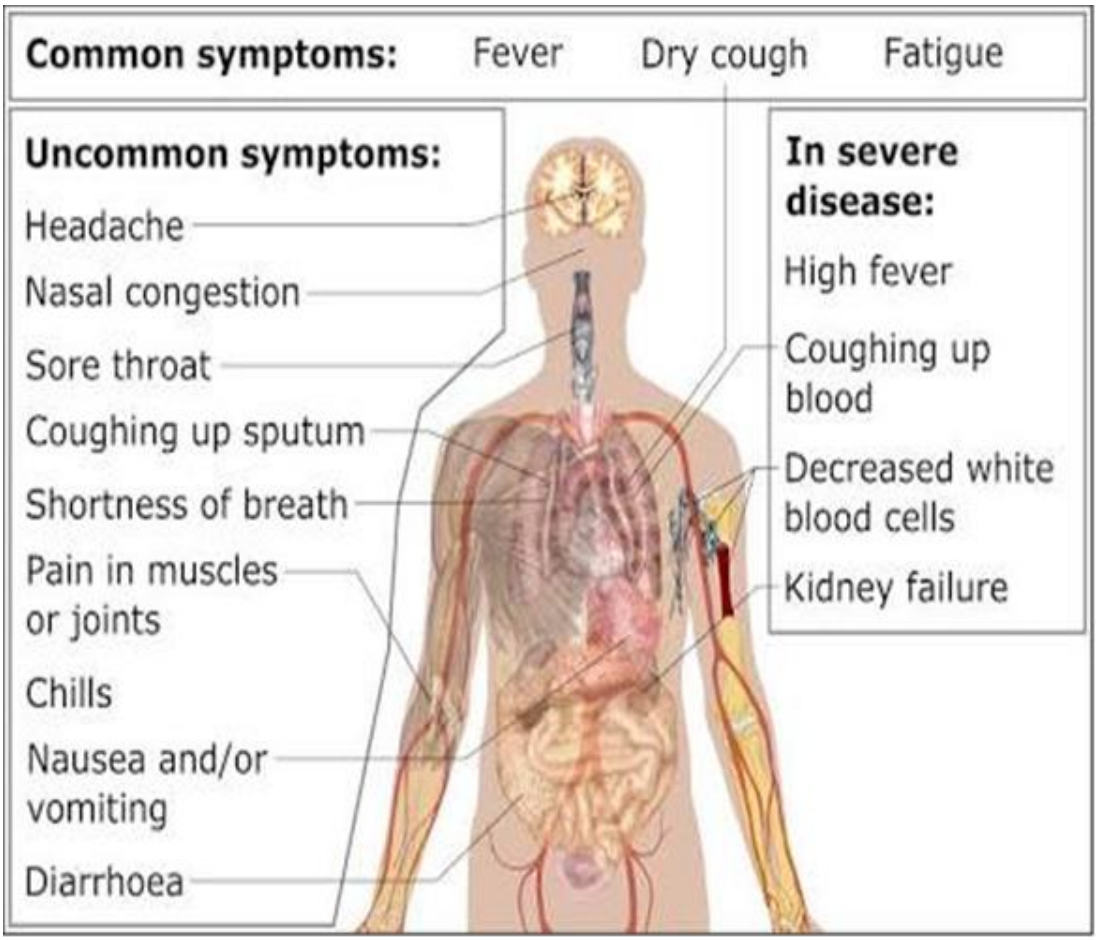

Fig. 6. Common and uncommon symptoms of COVID-19

3.2 Preventive Actions and Spreading of COVID-19 to Others

- People should avoid close by contact with people sneezing and coughing and people that are sick.
- Avoid touching face, eyes, nose, and mouth with unwashed hands.
- Washing of hands regularly with soap and running water for at least 30 seconds. Also the use of alcohol-based hand sanitizer that contains at least 65% alcohol if soap and water are not available.
- Stay home when you are sick.
- People with COVID-19 symptoms produce a respiratory droplets when they cough or sneezes so, should practice cough manners or etiquette i.e maintaining distance when coughing or sneezing, cover coughs, wear face mask, sneezes with disposable tissues or clothing, and wash hands regularly.
- Clean and disinfect frequently all touched objects and surfaces.
- Limit person-to-person transmission which include reducing secondary infections amidst close by contacts and health care workers, and preventing transmission amplification.
- Identify, isolate and be concerned for patients timely, including providing enhance care for infected patients to avoid spread of the disease.
- Avoid unprotected contact with wild animals to Identify and reduce spread of virus from the animal source.
- Converse critical risk and event information to all communities and counter misinformation.
- Address all crucial unknowns regarding clinical harshness, extent of transmission and infection treatment options, and accelerate the development of diagnostics, therapeutics and vaccines.
- Minimize social and economic impact through multispectral partnerships.
- People that have traveled from an affected area, should be isolated and restrictions for 2 weeks. If the patient develop symptoms during the isolation period with fever, cough, and trouble breathing, then seek for medical advice or call the health care provider for COVID-19 in the community before telling them about the travel and the symptoms.

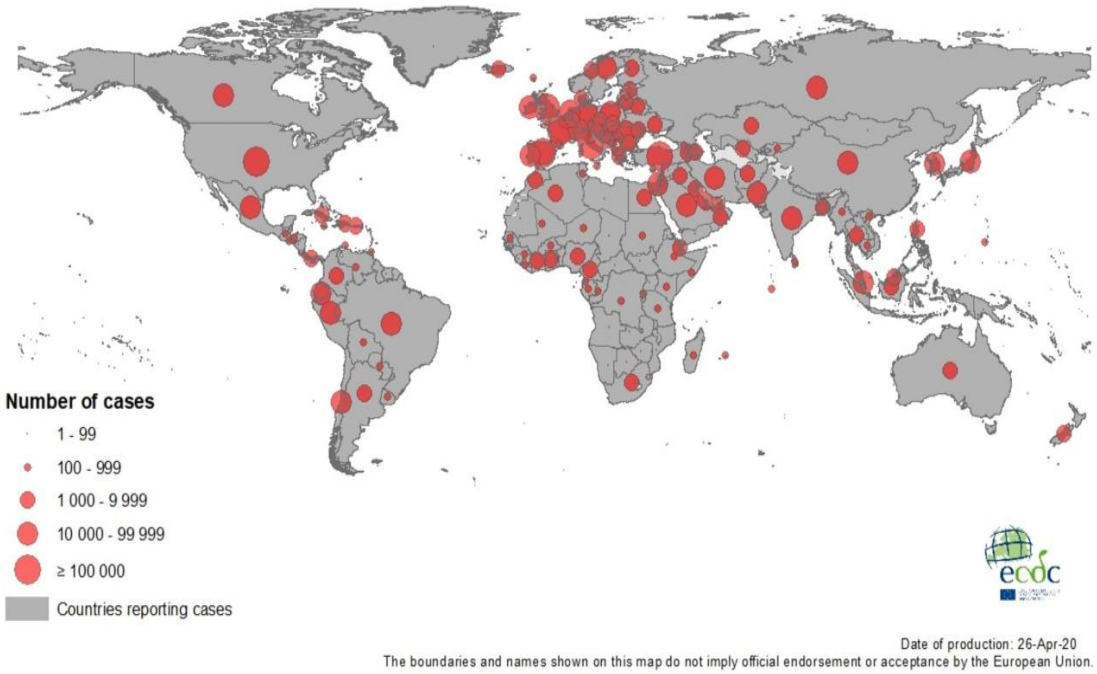

Fig. 7. Countries areas with reported confirmed cases of COVID-19, 26 April 2020 by ECDC

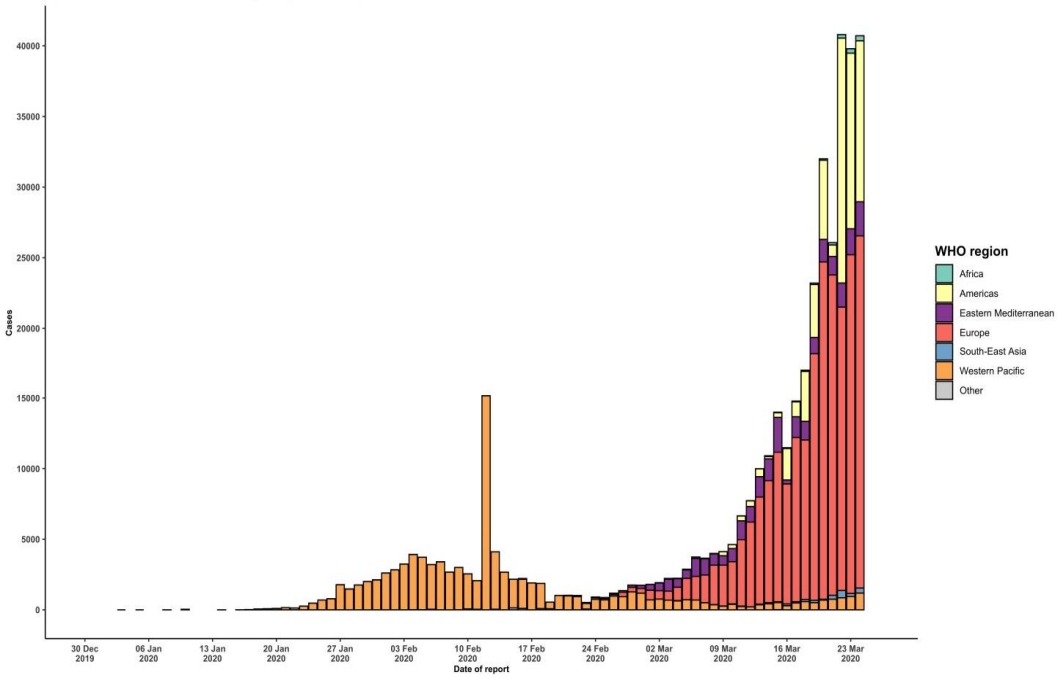

Fig. 8. Epidemic curve of confirmed COVID-19, by date of report and WHO region through 25 March 2020

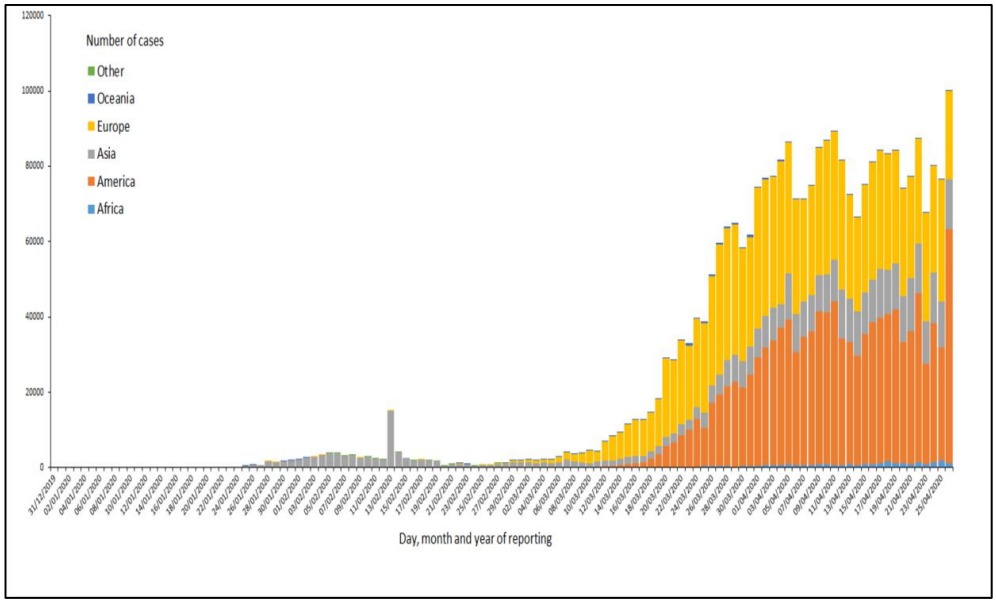

Fig. 9. Distribution of COVID-19 cases worldwide, as at 26 April, 2020 by ECDC

3.3 Summary Situation Worldwide as at 26 April 2020 Reported by ECDC [38]

- **Globally:** 2,931,787 confirmed cases worldwide and 203,596 deaths worldwide.
- **Asia:** 456350 confirmed, and most case were reported in Turkey, Iran, China, India and Saudi Arabia with 107773, 89328, 83909, 26496, and 16299 respectively and 16865 deaths, most death are in Iran,

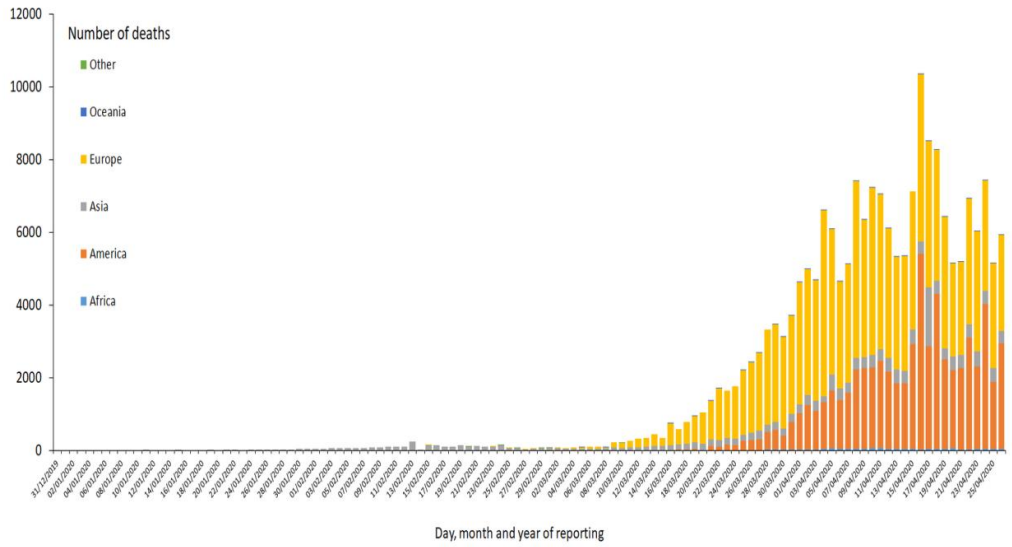

Fig. 10. Distribution of COVID-19 death cases worldwide, as at 26 April, 2020 by ECDC

China, Turkey, India and Indonesia with 5650, 4636, 2706, 824 and 720 death respectively.

- **America:** 1134686 confirmed, most reported countries are US, Brazil, Canada, Peru and Mexico with 939053, 58509, 45341, 25331 and 13842 respectively and 63649 deaths, most death were recorded in US, Brazil, Canada, Mexico and Peru with 53189, 4016, 2465, 1305 and 700 death respectively.
- **Europe:** 1214584 confirmed, five most confirm case were in Spain, Italy, Germany, United kingdom and France with 219764, 195351, 154175, 148377 and 124114 respectively and 119306 deaths, most death were recorded in Italy, France, Spain, United kingdom and Belgium with 26384, 22614, 22524, 20319 and 6917 death respectively.
- **Oceania:** 8080 confirmed, five most cases were in Australia, New Zealand, Guam, Frence Polynesia and Fiji with 6703, 1121, 141, 57, 18 death respectively and 106 deaths, with the most death case recorded in Australia, New Zealand, Guam and Northern Mariana islands with 81, 18, 5, 2 death respectively.
- **African Region:** 30316 confirmed cases, five most countries are South Africa, Egypt, morocco, Algeria and Cameroon with 4361, 4319, 3897, 3256 and 1518 respectively and 1382 deaths, the top most countries are Algeria, Egypt, morocco, south Africa and Cameroon with 419, 307, 159, 86 and 53 death cases respectively
- **Others:** 696 confirmed and 7 deaths

4. TREATMENT

4.1 Efficacy and Safety of Chloroquine, Remdesivir (GS-5734) and Hydroxy-chloroquine for the Treatment of COVID-19

Coronavirus Disease-2019 (COVID-19) is a public health emergency of international concern. As of this time there is no recognize exact, effectual, proven, pharmacological treatment for the cure of COVID-19. Invitro studies have suggested that chloroquine, an immune modulant drug traditionally used to cure malaria, is effective in reducing viral replication in other contagion, as well as the SARS-associated coronavirus (CoV) and MERS-CoV [39,40]. Chloroquine (N4-(7-Chloro-4-quinolinyl)-N1,N1-diethyl-1,4-pentanediamine) (CQ) has been used worldwide for above 80 years, and it is part of the World Health Organization (WHO) model list of vital medicines. The drug is also inexpensive and has a set up clinical safety profile. However, the effectiveness and safety of the drug chloroquine for treatment of SARS-CoV-2 (the novel virus causing COVID-19) pneumonia remains indistinct.

A group of Chinese researchers, from literature have studied the impact of chloroquine in vitro, using Vero E6 cells contaminated by SARS-CoV-2 at an array of contagion (MOI) of 0.05. The study established the fact that chloroquine was extremely effectual in decrease viral N protein replication, with an effectual dosage (EC) 90 of 6.90 μM that can be easily attainable with normal standard dosing, due to its advantageous penetration in tissues, including in the lung. The authors described that chloroquine is known to obstruct virus contagion by cumulating endosomial pH and by meddling with the glycosylation of cellular receptor of SARSCoV. Also guesswork on the possibility that the known immunomodulant effect of the drug may improve the antiviral effect *in vivo* [41].

A description letter written by Chinese authors give an account that a news meeting from the State Council of China had stated that chloroquine phosphate had established marked effectiveness and satisfactory safety in treating COVID-19 related pneumonia in multi-center clinical trials conducted in China. The authors also specified that these discovery came from more than 100 patients included in the trials [42]. Evidence of such data was sought in the trial registries to review and was found none. So far cases had been reported in 213 countries as at 26th April 2020, the low cost of chloroquine is a main advantage for both the extreme stressed healthcare systems of involved elevated-income countries and none funded healthcare systems of mid-and low-income counties.

The skilled agreement was in print on 20th February by a multicentre cooperation group of the Department of Science and Technology of Guangdong Province and Health Commission of Guangdong Province paper and have to do with specifically to the use of chloroquine phosphate. No information was provided on condition that the method used to attain agreement [43]. Based on the in vitro evince and still not published clinical experience, the board recommended and endorsed chloroquine phosphate tablet, at a dose of 500 mg twice per day for 10 days, for patients diagnosed as slight mild and severe cases of SARS-CoV-2 pneumonia, on condition that there were no opposition or contraindication to the drug.

Research have shown that chloroquine (CQ) and remdesivir (GS-5734), efficiently inhibited SARS-CoV-2 contagious in vitro. Remdesivir is a nucleoside analog pro drug technologically advanced by Gilead Sciences (USA). A clinical report in United States showed that treatment with remdesivir improved and better the clinical state of the first patient infected by SARS-CoV-2 [44] and a stage III clinical trial of remdesivir in opposition to SARSCoV-2 was open in Wuhan on February 4, 2020. Although, as an experimental drug, remdesivir is not likely to be largely obtainable for treating a very large number of patients in a timely way. However, of the two potential drugs for treating of COVID-19, CQ happen to be the best drug of choice for large-scale use because of its obtainability and availability, a safety record proven, and a moderately low in cost.

Chloroquine CQ (N4-(7-Chloro-4-quinolinyl)-N1, N1-diethyl-1,4-pentanediamine) have been in used for long in the treatment of malaria and amebiasis. However, *Plasmodium falciparum* technologically advanced extensive resistance to it, and with the advancement of new anti-malarial drugs, CQ has become a choice for the prophylaxis of malaria. Furthermore, over dose of CQ can cause acute or severe poisoning and death [45]. In the past years, due to occasional use of CQ in clinical practice, its manufacture and market supply was very much reduced, at least in China.

Hydroxychloroquine (HCQ) sulfate, is a derivative of CQ, and was first synthesized in 1946 by initiating a hydroxyl group OH into CQ. HCQ establish and demonstrated to be much less (~40%) toxic than CQ in animals [46]. HCQ is still extensively obtainable and available to treat autoimmune diseases, such as rheumatoid arthritis and systemic lupus erythematosus. Since CQ and HCQ have similar chemical structures and mechanisms of acting as a feeble base and immune modulator, HCQ have shown to be more effective and potent drugs to treat SARS-CoV-2. In fact, as of February 23, 2020, seven clinical trial records were found in Chinese Clinical Trial Registry for using HCQ to treat SARS-CoV-2. What makes HCQ to be more efficacious than CQ in treating SARS-CoV-2 infection still be short of experimental prove.

Liu et al. [47] assess the antiviral impact of HCQ against SARS-CoV-2 infection in comparison to CQ in vitro. Initially, the cytotoxicity of HCQ and CQ in African green monkey kidney VeroE6 cells (ATCC-1586) was determined by standard CCK8 assay. The result demonstrated that the 50% cytotoxic concentration (CC50) values of CQ and HCQ were 273.20 and 249.50 μM, respectively,

which shows a no significant different from each other. The antiviral activity of CQ versus HCQ was compared, and the dose–response curves of the two compounds against SARS-CoV-2 were evaluated at four several multiplicities of infection (MOIs) by enumerate of viral RNA copy numbers in the cell supernatant at 48 h post infection (p.i.). The result showed that, at all MOIs (0.01, 0.02, 0.2, and 0.8), the 50% maximal effectiual concentration (EC50) for CQ were 2.71, 3.81, 7.14 and 7.36 μM respectively, and HCQ were 4.51, 4.06, 17.31, and 12.96 μM respectively at all MOIs. CQ values was lower than that of HCQ. The differences in EC50 values were statistically significant at an MOI of 0.01 ($P < 0.05$) and MOI of 0.2 ($P < 0.001$).

Consequently, the selectivity index (SI = CC50/EC50) of CQ (100.81, 71.71, 38.26, and 37.12) was higher than that of HCQ (55.32, 61.45, 14.41, 19.25) at MOIs of 0.01, 0.02, 0.2, and 0.8 respectively. These results were verify by immune fluorescence microscopy as indication by several expression stage of the virus nucleoprotein (NP) at the indicated drug concentrations at 48 h p.i. This study suggest that the anti-SARS-CoV-2 activity of HCQ appear to be less strong or potent compared to CQ, at slightest at certain MOIs. The two drugs CQ and HCQ are feeble bases that are known to raise the pH of acidic intracellular organelles, such as endosomes/lysosomes, vital for membrane synthesis.

Furthermore, CQ could inhibit SARS-CoV entry via altering the glycosylation of ACE2 receptor and spike protein [48]. Extra additional experiment confirmed that HCQ efficiently inhibited the entry step, in addition to the post-entry stages of SARS-CoV-2, which was also found upon CQ treatment.

To further discover the full mechanism of action of CQ and HCQ in inhibiting the COVID-19 entry, co-localization of virions with early endosomes (EEs) or endo lysosomes (ELs) was examine by immunofluorescence analysis (IFA) and confocal microscopy. Quantification examination showed that, at 90 min p.i. in untreated cells, 16.2% of adopt virions (anti-NP, red) were detected in early endosome antigen 1 (EEA1)-positive EEs (green), whereas more virions (34.3%) were transfer into the late endosomal–lysosomal protein LAMP1+ ELs (green) ($n > 30$ cells for each group). By difference, in the presence of CQ or HCQ, significantly more virions (35.3% for CQ and 29.2% for HCQ; shows a significant $P < 0.001$) were detected in the EEs, while only very few virions (2.4% for CQ and 0.03% for HCQ; $P < 0.001$) were found to be co-localized with LAMP1+ ELs ($n > 30$ cells).

This suggested that both CQ and HCQ obstruct the transport of SARS-CoV-2 from EEs to ELs, which happen to be a condition to release the viral genome as in the case of SARS-CoV [49]. However, CQ and HCQ treatment caused obvious changes in the number and size/morphology of EEs and ELs. In CQ and HCQ-treated cells, unusually increase EE vesicles were seen, arrows in the upper panels), many of which are even larger than ELs in the untreated cells. In the untreated cells, most EEs were much smaller than ELs. Previous report study have showed that treatment with CQ induced the formation of expanded cytoplasmic vesicles [50].

Within the EE vesicles, virions (red) were contained around the membrane (green) of the vesicle. The treatment with CQ did not cause noticeable changes in the number and size of ELs; however, the regular vesicle structure seemed to be disrupted, at least partially. By difference, in HCQ-treated cells, the size and number of ELs raised significantly, arrows in the lower panels. Since acidification is important and vital for endosome maturation and function, then surmise that endosome maturation might be blocked at midway stages of endocytosis, resulting in failure of additional transport of virions to the ultimate releasing site. CQ was reported to elevate the pH of lysosome from about 4.5 to 6.5 at 100 μM [51].

Research have shown that oral absorption of CQ and HCQ in humans is very effective in the treatment of SARS-CoV-2. In animals, both drugs share similar tissue distribution patterns, with high concentrations in the liver, spleen, kidney, and lung spread levels of 200–700 times higher than those in the plasma [52]. It was reported that safe dosage (6–6.5 mg/kg per day) of HCQ sulfate could generate serum levels of 1.4–1.5 μM in Humans. Therefore, with a safe recommended dosage, HCQ concentration in the above tissues is likely to be achieved to inhibit SARS-CoV-2 infection.

5. CONCLUSION

Coronaviruses are diverse family of viruses that interact at various levels with constituent of host cells taking this benefit of some of the cellular

machineries for replication and proliferation. In conclusion, CQ and HCQ can efficiently inhibit SARS-CoV-2 infection in vitro and it is more effective with HCQ. In combination with its anti-inflammatory function, and was predict that the drug has a good effect to combat the virus disease. This possibility awaits confirmation by clinical trials. HCQ is less toxic than CQ, prolonged and overdose usage of HCQ can still cause poisoning. Although the use of chloroquine can also support expert opinion, clinical use of this drug in patients with COVID-19 should stick to the ethical approval as a trial as stated by the WHO.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Coronavirus Disease (COVID-2019) Situation Reports 1−45; World Health Organization; 2020. Available:https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports
2. Anthony SJ, Johnson CK, Greig DJ, Kramer S, Che X, Wells H, Hicks AL, Joly DO, Wolfe ND, Daszak P, Karesh W, Lipkin WI, Morse SS, Mazet JAK, Goldstein T. Global patterns in coronavirus diversity. Virus Evol. 2017;3(1):vex012.
3. De Haan CA, Rottier PJ. Molecular interactions in the assembly of coronaviruses. Adv Virus Res. 2005;64: 165-230.
4. Masters PS. The molecular biology of coronaviruses. Adv Virus Res. 2006;66: 193-292.
5. Tan YJ, Lim SG, Hong W. Characterization of viral proteins encoded by the SARS-coronavirus genome. Antiviral Res. 2005; 65:69-78.
6. Chinese SMEC. Molecular evolution of the SARS coronavirus during the course of the SARS epidemic in China. Science. 2004; 303:1666-1669.
7. Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, Graham BS, McLellan JS. Cryo-EM structure of the 2019-nCoV Spike in the prefusion conformation. Science. 2020;eabb2507.
8. Hoffmann M, Kleine-Weber H, Schroeder S, Kruger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu NH, Nitsche A, Muller MA, Drosten C, Pohlmann S. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a Clinically Proven Protease Inhibitor. Cell; 2020. DOI: 10.1016/j.cell.2020.02.052
9. Gorbalenya AE, Snijder EJ, Ziebuhr J. Virus-encoded proteinases and proteolytic processing in the Nidovirales. J. Gen. Virol. 2000;81(4):853−879.
10. Baez-Santos YM St. John SE, Mesecar AD. The SARScoronavirus papain-like protease: Structure, function and inhibition by designed antiviral compounds. Antiviral Res. 2015;115:21−38.
11. Niemann H, Geyer R, Klenk HD, Linder D, Stirm S, Wirth M. The carbohydrates of mouse hepatitis virus (MHV) A59: structures of the O-glycosidically linked oligosaccharides of glycoprotein E1. EMBO J. 1984;3:665-670.
12. Narayanan K, Makino S. Characterization of nucleocapsid-M protein interaction in murine coronavirus. Adv Exp Med Biol. 2001;494:577-582.
13. Escors D, Ortego J, Enjuanes L. The membrane M protein of the transmissible gastroenteritis coronavirus binds to the internal core through the carboxy-terminus. Adv Exp Med Biol. 2001;494: 589-593.
14. Raamsman MJB, Locker JK, de Hooge A, de Vries AA, Griffiths G, Vennema H, et al. Characterization of the coronavirus mouse hepatitis virus strain A59 small membrane protein E. J Virol. 2000;74:2333-2342.
15. Vennema H, Godeke GJ, Rossen JW, Voorhout WF, Horzinek MC, Opstelten DJ, et al. Nucleo capsid-independent assembly of coronavirus-like particles by co-expression of viral envelope protein genes. EMBO J. 1996;15:2020-2028.
16. De Diego ML, Alvarez E, Almazan F, Rejas MT, Lamirande E, Roberts A, et al. A severe acute respiratory syndrome coronavirus that lacks the E gene is

attenuated in vitro and *in vivo*. J Virol. 2007;81:1701-13.
17. Kuo L, Masters PS. The small envelope protein E is not essential for murine coronavirus replication. J Virol. 2003;77: 4597-4608.
18. Lai MM, Cavanagh D. The molecular biology of coronaviruses. Adv Virus Res. 1997;48:1-100.
19. Zirkel F, Kurth A, Quan PL, Briese T, Ellerbrok H, Pauli G, et al. An insect nidovirus emerging from a primary tropical rainforest. M Bio. 2011;2:e00077-11.
20. Gorbalenya AE, Enjuanes L, Ziebuhr J, Snijder EJ. Nidovirales: Evolving the largest RNA virus genome. Virus Research. 2006;117:17-37.
21. Su S, Wong G, Shi W, Liu J, Lai ACK, Zhou J, Liu W, Bi Y, Gao GF. Epidemiology, genetic recombination, and pathogenesis of coronaviruses. Trends Microbiol. 2016;24(6):490−502.
22. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P, Zhan F, Ma X, Wang D, Xu W, Wu G, Gao GF, Tan WA. Novel coronavirus from patients with pneumonia in China, 2019. N. Engl. J. Med. 2020;382(8): 727−733.
23. Tang B, Bragazzi NL, Li Q, Tang S, Xiao Y, Wu J. An updated estimation of the risk of transmission of the novel coronavirus (2019-nCov). Infect Dis Model. 2020;5: 248−255.
24. Susanna KPL, Kenneth SML, Alan KLT, Chung Tong S, Ming Wang, Garnet, Choi KV, et al. Recent Transmission of a Novel Alphacoronavirus, Bat Coronavirus HKU 10, from Leschenault's Rousettes to Pomona Leaf- Nosed Bats: First Evidence of Inter species Transmission of Coronavirus between Bats of Different Suborders. J Virol. 2012;86:11906-11918.
25. Bosch BJ, vander Zee R, de Haan CA, Rottier PJ. The coronavirus spike proteinisa class Ivirusfusionprotein: Structural and functional characterization of the fusion corecomplex. J Virol. 2003; 77:8801-8811.
26. Susan RW, Sonia N. Coronavirus pathogenesis and the emerging pathogen severe acute respiratory syndrome coronavirus. Microbiol Mol Biol Rev. 2005; 69:635-664.
27. Baranov PV, Henderson CM, Anderson CB, Gestel RF, Atkins JF, Howard MT. Programmed ribosomal frame shifting in decoding the SARS-CoV genome. Virology. 2005;332:498-510.
28. Cynthia SG, Kathleen MT, Thomas GK, Pierre ER, James AC, et al. Ultra structural characterization of SARS coronavirus. Emerg Infect Dis. 2004;10:320-326.
29. Gosert R, Kanjanahaluethai A, Egger D, Bienz K, Baker SC. RNA replication of mouse hepatitis virus takes place at double-membrane vesicles. J Virol. 2002; 76:3697-708.
30. Miller S, Krijnse-Locker J. Modification of intracellular membrane structures for virus replication. Nat Rev Microbiol. 2008;6: 363-74.
31. Mark RD. Seeking membranes: Positive-strand RNA virus replication complexes. PLoS Biol. 2008;6:e270.
32. Stertz S, Reichelt M, Spiegel M, Kuri T, Martinez Sobrido L, Garcia Sastre A, et al. The intracellular sites of early replication and budding of SARS-coronavirus. Virology. 2007;361:304-15.
33. Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, Graham BS, McLellan JS. Cryo-EM structure of the 2019-nCoV Spike in the prefusion conformation. Science. 2020;eabb2507.
34. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, Wang W, Song H, Huang B, Zhu N, Bi Y, Ma X, Zhan F, Wang L, Hu T, Zhou H, Hu Z, Zhou W, Zhao L, Chen J, Meng Y, Wang J, Lin Y, Yuan J, Xie Z, Ma J, Liu WJ, Wang D, Xu W, Holmes EC, Gao GF, Wu G, Chen W, Shi W, Tan W. Genomic characterization and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding; 2020.
35. Morse JS, et al. Learning from the past: Possible urgent prevention and treatment options for severe acute respiratory infections caused by 2019-nCoV. Chem BioChem. 2020;21(5):730−738.
36. Chan JFW, Kok KH, Zhu Z, Chu H, To KKW, Yuan S, Yuen KY. Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. Emerging Microbes Infect. 2020;9(1):221−236.
37. Dong N, et al. Genomic and protein structure modelling analysis depicts the origin and infectivity of 2019-nCoV, a new coronavirus which caused a pneumonia outbreak in Wuhan, China, bioRxiv; 2020.
DOI: 10.1101/2020.01.20.913368

38. European centre for disease prevention and control. Available:https://www.ecdc.europa.eu/en/geographical-distribution-2019-ncov-case
39. Savarino A, Boelaert JR, Cassone A, Majori G, Cauda R. Effects of chloroquine on viral infections: an old drug against today's diseases? Lancet Infect Dis. 2003; 3:722–7.
40. Colson P, Rolain JM, Raoult D. Chloroquine for the 2019 novel coronavirus SARSCoV-2. Int J Antimicrob Agents. 2020;105923.
41. Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) *in vitro*. Cell Res; 2020. Available:https://doi.org/10.1038/s41422–020–0282–0
42. Gao J, Tian Z, Yang X. Breakthrough: chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. Biosci Trends; 2020. Available:https://doi.org/10.5582/bst.2020.01047
43. Wu Zhong2, Manli Wang1. Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitroCell Discovery. Cell Discovery. 2020;6:16. Available:https://doi.org/10.1038/s41421-020-0156-0
44. Holshue ML, et al. First case of 2019 novel coronavirus in the United States. N. Engl. J. Med; 2020. Available:https://doi.org/10.1056/NEJMoa2001191
45. Weniger H. Review of side effects and toxicity of chloroquine. Bull. World Health. 1979;79:906.
46. McChesney EW. Animal toxicity and pharmacokinetics of hydroxychloroquine sulfate. Am. J. Med. 1983;75:11–18.
47. Liu Jia 1, Ruiyuan Cao2, Mingyue Xu1,3, Xi Wang1, Huanyu Zhang1,3, Hengrui Hu1,3, Yufeng Li1,3, Zhihong Hu 1. Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-COV-2 infection *in vitro*. Cell Discovery. 2020;6(16).
48. Savarino A, et al. New insights into the antiviral effects of chloroquine. Lancet Infect. Dis. 2006;6:67–69.
49. Mingo RM, et al. Ebola virus and severe acute respiratory syndrome coronavirus display late cell entry kinetics: evidence that transport to NPC1+ endolysosomes is a rate-defining step. J. Virol. 2015;89: 2931–2943.
50. Zheng N, Zhang X, Rosania GR. Effect of phospholipidosis on the cellular pharmacokinetics of chloroquine. J. Pharmacol. Exp. Ther. 2011;336:661–671.
51. Ohkuma S, Poole B. Fluorescence probe measurement of the intralysosomal pH in living cells and the perturbation of pH by various agents. Proc. Natl Acad. Sci. USA. 1978;75:3327–3331.
52. Popert AJ. Choloroquine: A review. Rheumatology. 1976;15:235–238.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/56272