




Hydroxychloroquine and Covid-19: A Cellular and Molecular Biology Based Update

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Received: 22 May 2020 / Accepted: 3 June 2020 / Published online: 10 June 2020
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Abstract As the time for finding a definitive and safe cure as a vaccine for novel Corona Virus Disease 2019 (Covid-19) is still far, there is need to study in depth about the other potential drugs, which can save millions of lives due to Covid-19 pandemic. Right at the center of the debate is the use of drug “Hydroxychloroquine” as a prophylaxis as well as a treatment strategy against Covid-19 in conjunction with azithromycin. In this review, we will study the cellular and molecular aspects of hydroxychloroquine, which had driven its use in Covid-19 patients, as well as its chemistry and pharmacokinetics along with clinical trials going on worldwide using hydroxychloroquine against Covid-19.

Keywords Covid-19 · Hydroxychloroquine · Cytokine storm · Severe acute respiratory syndrome coronavirus 2

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Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s12291-020-00900-x>) contains supplementary material, which is available to authorized users.

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Introduction

Without a therapeutic vaccine or specific antiviral drugs, and with a desperate attempt to find a cure against novel Corona Virus Disease 2019 (Covid-19) [1], the limelight was shifted to hydroxychloroquine (derivative of chloroquine that has antimalarial, antiinflammatory, immunosuppressive and autophagy activities [2, 3]; upon a tweet by US president Mr. Donald J. Trump. On 21st March, 2020, he tweeted, “Hydroxychloroquine and Azithromycin, taken together, have a real chance to be one of the biggest game changers in the history of medicine” (<https://twitter.com/realdonaldtrump/status/1241367239900778501?lang=en>).

However, easier said than done, initial studies of Covid-19 patients treated with hydroxychloroquine with or without azithromycin were shown to have mixed results, with more scrutiny being raised over the lack of proper design of these studies. Without clinical trials and robust methodologically correct scientific studies there is skepticism over benefits and efficacy of hydroxychloroquine against Covid-19. Till the writing of this article, as per the information available on NCBI, currently worldwide 1450 clinical trials are going on (<https://clinicaltrials.gov/ct2/results?cond=%22wuhan+coronavirus%22>) and 180 different compounds are being tested against Covid-19 (<https://pubchem.ncbi.nlm.nih.gov/#tab=compound&query=covid-19%20clinicaltrials>).

Though the exact mechanisms are still unclear; however, it has been established from scientific literature that at the molecular level, hydroxychloroquine affects the activity of lysosomes and in turn autophagy and disrupts lysosomal membrane stability. Also, it alters the transcriptional activity and signaling pathways. At the cellular level also, the precise mode of action of hydroxychloroquine is yet to

be delineated. Based upon current understanding, hydroxychloroquine has been shown to inhibit immune system activation. Suppression of immune system activation by hydroxychloroquine is achieved by reducing cytokine production and Toll-like receptor (TLR) signaling pathways [2, 3].

The main aim of this review is to discuss the mode of action of hydroxychloroquine at cellular and molecular levels, that potentially support the clinical efficacy and few adverse side effects observed in Covid-19 patients treated with hydroxychloroquine, which may further help in improving the clinical outcomes by modifying or altering the drug itself or its restricted use in certain individuals by enforcing strict inclusion and exclusion criteria. The readers are referred to more holistic review for clinical use of hydroxychloroquine in other diseases like rheumatoid arthritis, systemic lupus erythematosus, antiphospholipid syndrome and primary Sjögren syndrome [2, 3].

Covid-19

Towards the end of year 2019 (December, 2019), Chinese Province of Wuhan reported the emergence of Covid-19 in humans characterized with atypical pneumonia, fever, dry cough and progressive dyspnea [4]. Novel coronavirus 2019 (nCoV-2019), officially known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was demonstrated to cause Covid-19 [5]. Within a span of around 3 months, Covid-19 spread worldwide forcing the World Health Organization (WHO) to declare it as pandemic on 11th March 2020. As per the WHO-Covid-19 dashboard available at <https://covid19.who.int/>, as of 31st May, 2020, there have been 5,939,234 confirmed cases of Covid-19, including 367,255 deaths worldwide. It is now well established that respiratory transmission and direct contact transmission are the main routes for Covid-19 transmission. Previously, Coronaviruses (CoVs) also have been responsible for the severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) outbreaks. [6, 7].

Clinical Features and Laboratory Diagnosis of Covid-19

According to Centers for Disease Control and Prevention (CDC), USA (available at <https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms.html>) and recent literature, Covid-19 infected people demonstrate a wide range of symptoms—ranging from mild symptoms to severe illness. Most common symptoms of confirmed positive Covid-19 patients were fever, cough, shortness of

breath, myalgia or fatigue and sore throat; whereas, headache, chills, new loss of taste or smell, sputum production, diarrhea, and vomiting like symptoms may also occur [8–12].

Elderly people and people, who have severe underlying medical conditions, seem to be at higher risk for developing more serious complications from COVID-19 compared to young healthy adults [9, 10, 13, 14]. Most of deaths due to Covid-19 were due to severe respiratory failure, pneumonia and multiple organ failure [10].

Initially, a combination of computed tomography (CT) imaging, electron microscopy, and whole genome sequencing were used to screen and identify SARS-CoV-2 [4, 15–19]. Confirmatory diagnosis of Covid-19 relies on the real-time reverse transcriptase-polymerase chain reaction (RT-PCR) method. RNA-dependent RNA polymerase (RdRp) gene of the ORF1ab sequence, E gene, N gene, and S gene of the SARS-CoV-2 genome is the basis of RT-PCR tests for the diagnosis of SARS-CoV-2 [4, 15, 16].

Among the laboratory parameters, most moderate Covid-19 patient's exhibited leucopenia, lymphopenia, high level of C-reactive protein (CRP) and erythrocyte sedimentation rate [12, 20]. However, severe Covid-19 patients showed frequent lymphopenia, with increased levels of lactate dehydrogenase, CRP, ferritin, alanine aminotransferase, and D-dimer along with grossly higher levels of interleukins (ILs) IL-2R, IL-6, IL-10, and Tumor necrosis factor- α [9, 12, 14, 20]. Acute respiratory distress syndrome (ARDS) and multiple-organ failure in Covid-19 are thought to result from "Cytokine storm" also known as cytokine release syndrome. "Cytokine storm" is an excessive immune response to an external stimulus. "Cytokine storm" in severe Covid-19 cases, if left untreated for a longer time, results in an increased risk of vascular hyperpermeability, multiorgan failure, and eventually death [21, 22]. Due to observance of Cytokine storm in severe Covid-19 cases; some authors have suggested theoretical treatment strategies like use of IL-6 and IL-1 family antagonists, TNF blockers and corticosteroid therapy among other strategies [22, 23].

Hydroxychloroquine: Structure and Its Chemistry

Hydroxychloroquine, (PubChem CID: 3652, <https://pubchem.ncbi.nlm.nih.gov/compound/3652>, Molecular formula: $C_{18}H_{26}ClN_3O$) also known as Plaquenil, Oxichloroquine, Oxychlorochin, is a derivative of chloroquine exhibiting immunosuppressive, anti-inflammatory, antiautophagy, and antimalarial activities. Due to these properties, hydroxychloroquine is now most often used as an antirheumatologic agent in systemic lupus erythematosus and rheumatoid arthritis. This is the reason that both

hydroxychloroquine and chloroquine are classified under Disease-modifying antirheumatic drugs (DMARDs). Hydroxychloroquine is derived from chloroquine, and differ from it by the presence of a hydroxyl group. Both hydroxychloroquine and chloroquine are weak bases, and a half-life of around 50 days. Hydroxychloroquine and chloroquine belong to 4-aminoquinolines drugs class (reviewed in [3]).

Pharmacokinetics of Hydroxychloroquine

As chloroquine and hydroxychloroquine have almost similar structure and mode of action, both drugs can be used against SARS-CoV-1 and SARS-CoV-2 [24, 25]. As compared to chloroquine, hydroxychloroquine is a better therapeutic agent in terms of safety index. Various modes of action have been postulated to explain the therapeutic uses of hydroxychloroquine, but most of them are based on in vitro studies (reviewed in [3]). Hydroxychloroquine, which is administered as hydroxychloroquine sulfate, has a lag time of 0–0.85 h for 200 mg salt. It is the time between oral absorption and its measurement in the blood. It is usually absorbed in the upper intestinal region [26] and has an overall bioavailability of 0.7–0.8. The concentration of hydroxychloroquine can vary not only in plasma, blood and serum of individual patient but also among different patients [27]. It is intriguing to note that hydroxychloroquine has a half-life period of approximately 1–2 months and the mean residence time of nearly 1300 h [28].

Mode of Action of Hydroxychloroquine

The precise mechanism of action of hydroxychloroquine is not known till date, and there is lots of research going on the existing knowledge gap using diverse advanced scientific tools like [3] Computational biology [29], Immunology [30, 31], Structural biology [32, 33], Modern molecular medicine [34, 35], Synthetic biology [36, 37], and ‘big data’-driven public health science [38, 39].

Hydroxychloroquine may suppress immune function by interfering with the processing and presentation of antigens, and the production of cytokines. Acidic vesicles, such as endosomes/lysosomes, which are essential for the membrane fusion, act as major locations of action of chloroquine and hydroxychloroquine [2, 3, 40]. Chloroquine accumulates in lysosomes (lysosomotropism), and by increasing their pH, interferes with the glycosylation of cellular receptor of SARS-CoV-2 and thereby inhibits the infection of virus [14, 40]. An important feature of hydroxychloroquine is its capability to inhibit the innate as well as acquired immunity [41]. Hydroxychloroquine may

also affect signaling of TLR receptors by changing the endosomal pH [42] as well as by inhibiting TLR–ligand interactions. Further research is required to know the exact mechanism by which this drug affects TLR activation.

Additionally, hydroxychloroquine by interfering the lysosomal activity and autophagy, inhibits the antigen processing and presentation along with MHC-II molecules and subsequently immune system activation. Reduced activation of CD4⁺ T cells leads to decreased production of proinflammatory markers and cytokines [14, 40]. It has been suggested that autophagy is involved in autoantigen presentation mediated by MHC class II molecules, and activation of T-helper cells [43]. Because of immunomodulatory and anti-inflammatory properties, hydroxychloroquine can prove to be a better remedy for SARS-CoV-2 patients, where cytokine storm has emerged as a potentially fatal condition [12, 22].

As an anti-inflammatory agent, hydroxychloroquine is also used to treat various autoimmune disorders such as rheumatoid arthritis, systemic lupus erythematosus and Sjogren’s syndrome [3, 5]. Hydroxychloroquine also exhibits antibacterial property particularly against intracellular bacteria such as *Coxiella burnetii* [44]. However, due to strong affinity of hydroxychloroquine for melanin, it can deposit in tissues such as the skin and the eyes (hydroxychloroquine retinopathy) [3].

Hydroxychloroquine is thought to prevent the thrombotic complications by behaving like anticoagulant agents, and hence can prove beneficial for cases with a secondary coagulopathy owing to systemic inflammation [45]. This is done most probably by inhibiting the platelet aggregation [46, 47] or inhibition of binding of antiphospholipid antibody in autoinflammatory diseases [48]. Hydroxychloroquine is found to be safe to use during pregnancy and various studies have shown that it checks the initiation of congenital heart blockage [49, 50], possibly by reducing the expression of a type I interferon (IFN) signature [51].

Hydroxychloroquine interaction with other drugs

During combination therapy, hydroxychloroquine may interfere the functioning of other drugs or vice versa because it is metabolized by the same enzyme cytochrome P450 (CYP), which is responsible for the metabolism of many other drugs [3, 27, 52, 53]. Hydroxychloroquine increases the bioavailability of metoprolol in blood plasma by reducing its metabolism. Although similar effect is not observed for drugs, such as dextromethorphan [54] and methotrexate [55]. On the other hand, bioavailability of hydroxychloroquine may be affected by the medicines that increase the pH of gastric juice [3]. Hydroxychloroquine

can also interfere with drugs that affect the QT interval; hence possibility of cardiac arrhythmia should be checked in such patients [56, 57]. Combination therapy of breast cancer using tamoxifen and hydroxychloroquine may cause eye toxicity due to synergistic inhibition of enzymes in lysosomes of retinal epithelial cells [58]. Thus, duration of such therapy should be monitored carefully. Some of the adverse outcomes of hydroxychloroquine usage are gastrointestinal effects, including nausea, vomiting, diarrhea and abdominal discomfort [59]. Moreover, Hydroxychloroquine-associated cardiotoxic effects including rhythm disorders and cardiomyopathy in rheumatic patients have also been reported [57, 60].

Hydroxychloroquine and Covid-19

Hydroxychloroquine has attracted attention over the past few decades as a potential antiviral agent, and currently as a possible treatment for Covid-19 [40, 61, 62]. However, whether hydroxychloroquine works in vivo against Covid-19 remains to be seen conclusively. The word of caution is that in randomized controlled trials against a number of viruses, including influenza, hydroxychloroquine haven't work at all. Hydroxychloroquine is known to decrease the acidity in endosomes, which in turn might avert the endosome from releasing the virus into the cytoplasm. Biggest limitation is the lack of preclinical data for hydroxychloroquine against Covid-19 and its repeated failure against most viral diseases under in vivo conditions [61].

Another issue with hydroxychloroquine is adverse outcomes in certain patients with comorbid conditions who are on other medications [40, 61, 62]. It has been reported that hydroxychloroquine can also interfere with drugs that affect the QT interval [57]. One concern in the Covid-19 patients receiving hydroxychloroquine/chloroquine \pm azithromycin is the prolongation of QT interval, which may possibly increase the risk of Torsade de pointes (TdP; [63]) and sudden cardiac death. A prospective observational study of large cohort of COVID-19 patients treated with chloroquine/hydroxychloroquine \pm azithromycin has demonstrated no instances of TdP or arrhythmogenic death. Even though, it was shown that use of these medications resulted in QT prolongation [64].

Due to its cellular and molecular effects as discussed in previous sections, quite a few clinical trials are studying the effectiveness and safety of hydroxychloroquine (also chloroquine) for Covid-19 (<https://clinicaltrials.gov/ct2/results?cond=%22wuhan+coronavirus%22>). Upon searching for interventional studies among clinical trials against Covid-19, till the writing of this article we (authors) have

found 885 interventional studies using various drugs. Among these 885 studies, 51 studies were found to be using hydroxychloroquine alone (Table 1) and 104 studies are using hydroxychloroquine in combination with other antiviral/nutritional supplements/probiotics etc. (Supplementary material 1). Most of the studies for hydroxychloroquine against Covid-19 are focussing on its safety and efficacy as well its use as a prophylaxis measure against Covid-19 (Table 1). Few of these clinical trials are also evaluating optimal hydroxychloroquine dose, and its use in moderate and severe Covid-19 cases, and pregnant Covid-19 positive women (Table 1). Seven clinical trial registries were also found in Chinese Clinical Trial Registry (<http://www.chictr.org.cn>) for using hydroxychloroquine to treat Covid-19 [5].

In a recent significant development (on May 22nd, 2020), an observational study published in Lancet journal reported that hydroxychloroquine or chloroquine, when used alone or with macrolide, on in-hospital outcomes for Covid-19 were associated with decreased in-hospital survival along with an increased frequency of ventricular arrhythmias [65]. Acting swiftly on this report on May 27th, 2020, WHO executive group implemented a temporary pause of the hydroxychloroquine arm within the Solidarity Trial while the safety data is reviewed by the Data Safety Monitoring Board (<https://www.who.int/emergencies/diseases/novel-coronavirus-2019/global-research-on-novel-coronavirus-2019-ncov/solidarity-clinical-trial-for-covid-19-treatments>). Expressing shock over this move by WHO, various eminent scientists (> 140 scientists) have written an open letter to Editor of Lancet journal and authors of the study about their concerns over data integrity, plenty of loopholes and erroneous conclusions in the study among host of other concerns (<https://www.tropicalmedicine.ox.ac.uk/news/an-open-letter-to-richard-horton-editor-of-the-lancet-regarding-mehra-et-al>).

However, Indian Council of Medical Research (ICMR), New Delhi, India has maintained its earlier guidelines on use of hydroxychloroquine as prophylaxis for SARS-CoV-2 infection (<https://www.mohfw.gov.in/pdf/AdvisorontheuseofHydroxychloroquina sprophylaxisforSARSCoV2infection.pdf>) in its revised guidelines too (https://www.icmr.gov.in/pdf/covid/techdoc/V5_Revised_advisory_on_the_use_of_HCQ_SARS_CoV2_infection.pdf). In its revised guidelines published on 22nd May, 2020, ICMR has expanded to include following personnel for use of hydroxychloroquine as prophylaxis for SARS-CoV-2 infection (1) All asymptomatic healthcare workers working in Covid hospital/non-Covid hospitals/non-Covid areas of Covid hospitals/blocks (2) Asymptomatic frontline workers/paramilitary/police personnel working in Covid-19 containment zones, and (3)

Table 1 Clinical trials with hydroxychloroquine alone

S. no.	Clinical trial study title	Intervention (drug/s)	Weblink to clinical trial
<i>Hydroxychloroquine as prophylaxis drug against Covid-19</i>			
1	Prophylaxis for Patients at Risk of COVID-19 infection	Drug: Hydroxychloroquine Sulfate 200 MG	https://ClinicalTrials.gov/show/NCT04389359
2	Hydroxychloroquine Chemoprophylaxis in Healthcare Personnel in Contact With COVID-19 Patients (PHYDRA Trial)	Drug: HydroxychloroquineDrug: Placebo oral tablet	https://ClinicalTrials.gov/show/NCT04318015
3	Randomized, Controlled, Double-blind Clinical Trial Comparing the Efficacy and Safety of Chemoprophylaxis With Hydroxychloroquine in Patients Under Biological Treatment and/or JAK Inhibitors in the Prevention of SARS-CoV-2 Infection	Drug: HidroxicloroquinaDrug: Control group	https://ClinicalTrials.gov/show/NCT04330495
4	Prophylaxis of Exposed COVID-19 Individuals With Mild Symptoms Using chloroquine Compounds	Drug: Hydroxychloroquine Sulfate Regular doseDrug: Hydroxychloroquine Sulfate Loading DoseDrug: ChloroquineDrug: Placebo	https://ClinicalTrials.gov/show/NCT04351191
5	PROLIFIC Chemoprophylaxis Trial (COVID-19)	Drug: Hydroxychloroquine-Daily dosingDrug: Hydroxychloroquine-Weekly DosingOther: Matched Placebo Hydroxychloroquine	https://clinicaltrials.gov/show/NCT04352933
6	Immune Monitoring of Prophylactic Effect of Hydroxychloroquine in Healthcare Providers Highly Exposed to COVID-19	Drug: HydroxychloroquineDrug: Placebo oral tablet	https://ClinicalTrials.gov/show/NCT04346329
7	Pre-exposure Prophylaxis for SARS-Coronavirus-2	Drug: HydroxychloroquineOther: Placebo	https://ClinicalTrials.gov/show/NCT04328467
8	Post-Exposure Prophylaxis for Asymptomatic SARS-CoV-2 COVID-19 Patients With chloroquine Compounds	Drug: Hydroxychloroquine Sulfate Regular doseDrug: Hydroxychloroquine Sulfate Loading DoseDrug: ChloroquineDrug: Placebo	https://ClinicalTrials.gov/show/NCT04346667
9	Hydroxychloroquine as Prophylaxis for COVID-19 in Healthcare Workers (HCQPreP)	Drug: HydroxychloroquineDrug: Placebo	https://ClinicalTrials.gov/show/NCT04363450
10	Assessment of the Efficacy and Safety of (HCQ) as a Prophylaxis for COVID19 for Health Professionals	Drug: Hydroxychloroquine (HCQ)Drug: Placebo oral tablet	https://ClinicalTrials.gov/show/NCT04349228
11	Protect: Study With Hydroxychloroquine for Prevention and Early Phase Treatment of Coronavirus Disease (COVID-19)	Drug: Hydroxychloroquine	https://ClinicalTrials.gov/show/NCT04363827
12	Health Care Worker Prophylaxis Against COVID-19: The HERO Trial	Drug: Hydroxychloroquine Pre-Exposure ProphylaxisDrug: Placebo oral tablet	https://ClinicalTrials.gov/show/NCT04352946
13	Hydroxychloroquine as Chemoprevention for COVID-19 for High Risk Healthcare Workers	Drug: Hydroxychloroquine Sulfate (HCQ)	https://ClinicalTrials.gov/show/NCT04345653
14	Hydroxychloroquine Post Exposure Prophylaxis for Coronavirus Disease (COVID-19)	Drug: HydroxychloroquineDrug: Placebo oral tablet	https://ClinicalTrials.gov/show/NCT04345653

Table 1 continued

S. no.	Clinical trial study title	Intervention (drug/s)	Weblink to clinical trial
15	Pre-Exposure Prophylaxis With Hydroxychloroquine for High-Risk Healthcare Workers During the COVID-19 Pandemic	Drug: Hydroxychloroquine Drug: Placebos	https://ClinicalTrials.gov/show/NCT04318444
16	Prophylactic Benefit of Hydroxychloroquine in COVID-19 Cases With Mild to Moderate Symptoms and in Healthcare Workers With High Exposure Risk	Drug: Hydroxychloroquine Sulfate	https://ClinicalTrials.gov/show/NCT04371926
17	Hydroxychloroquine Chemoprophylaxis for COVID-19 Infection in High-risk Healthcare Workers.	Drug: Hydroxychloroquine Drug: Placebo oral tablet	https://ClinicalTrials.gov/show/NCT04370015
18	Hydroxychloroquine as Post Exposure Prophylaxis for SARS-CoV-2(HOPE Trial)	Drug: Hydroxychloroquine as post exposure prophylaxis	https://ClinicalTrials.gov/show/NCT04330144
19	Does Hydroxychloroquine Before & During Patient Exposure Protect Healthcare Workers From Coronavirus?	Drug: Hydroxychloroquine Drug: Placebo oral tablet	https://ClinicalTrials.gov/show/NCT04374942
20	Hydroxychloroquine to Prevent COVID-19 Disease Amongst Healthcare Workers	Drug: Apo-Hydroxychloroquine Drug: Matched Placebo	https://clinicaltrials.gov/show/NCT04371523
21	Hydroxychloroquine Versus Placebo in COVID-19 Patients at Risk for Severe Disease	Drug: Hydroxychloroquine Drug: Placebo	https://clinicaltrials.gov/show/NCT04325893
<i>Clinical trials on safety and efficacy of Hydroxychloroquine against Covid-19</i>			
22	The PATCH Trial (Prevention And Treatment of COVID-19 With Hydroxychloroquine)	Drug: Hydroxychloroquine Sulfate 400 mg twice a day Drug: Hydroxychloroquine Sulfate 600 mg twice a day Drug: Hydroxychloroquine Sulfate 600 mg once a day Drug: Placebo oral tablet	https://ClinicalTrials.gov/show/NCT04329923
23	ALBERTA HOPE COVID-19 for the Prevention of Severe COVID19 Disease	Drug: Hydroxychloroquine	https://ClinicalTrials.gov/show/NCT04329611
24	Hydroxychloroquine in SARS-CoV-2 (COVID-19) Pneumonia Trial	Drug: Hydroxychloroquine	https://clinicaltrials.gov/show/NCT04382625
25	A Pilot Study to Assess Hydroxychloroquine in Patients With SARS-CoV-2 (COVID-19)	Drug: Hydroxychloroquine Drug: Placebo	https://clinicaltrials.gov/show/NCT04363866
26	COVID-19 PrEP HCW HCQ	StudyDrug: Hydroxychloroquine (HCQ)	https://ClinicalTrials.gov/show/NCT04354870
27	Hydroxychloroquine in the Prevention of COVID-19 Infection in Healthcare Workers	Drug: Hydroxychloroquine	https://ClinicalTrials.gov/show/NCT04333225
28	Norwegian Coronavirus Disease 2019 Study	Drug: Hydroxychloroquine Sulfate	https://ClinicalTrials.gov/show/NCT04333225

Table 1 continued

S. no.	Clinical trial study title	Intervention (drug/s)	Weblink to clinical trial
29	Will Hydroxychloroquine Impede or Prevent COVID-19	Drug: Hydroxychloroquine-Daily Dosing Drug: Hydroxychloroquine-Weekly Dosing Other: Placebo oral	https://ClinicalTrials.gov/show/NCT04316377
30	A Study of Hydroxychloroquine Compared to Placebo as Treatment for People With COVID-19	Drug: Hydroxychloroquine Other: Placebo	https://ClinicalTrials.gov/show/NCT04379492
31	Efficacy and Safety of Hydroxychloroquine for Treatment of COVID-19	Drug: Hydroxychloroquine	https://ClinicalTrials.gov/show/NCT04261517
32	Chloroquine/Hydroxychloroquine Prevention of Coronavirus Disease (COVID-19) in the Healthcare Setting	Drug: Chloroquine or Hydroxychloroquine Drug: Placebo	https://ClinicalTrials.gov/show/NCT04303507
33	PATCH 2&3:Prevention & Treatment of COVID-19 (Severe Acute Respiratory Syndrome Coronavirus 2) With Hydroxychloroquine	Drug: Group A HCQ Drug: Group B Control	https://ClinicalTrials.gov/show/NCT04353037
34	Hydroxychloroquine Treatment of Healthcare Workers With COVID19 Illness at Montefiore	Drug: Hydroxychloroquine	https://ClinicalTrials.gov/show/NCT04350450
35	Healthcare Worker Exposure Response and Outcomes of Hydroxychloroquine	Drug: Hydroxychloroquine Drug: Placebo oral tablet	https://ClinicalTrials.gov/show/NCT04334148
36	Safety And Efficacy Of Hydroxychloroquine For At Risk Population (SHARP) Against COVID-19	Drug: Hydroxychloroquine Sulfate 200 mg (mg) Tab	https://ClinicalTrials.gov/show/NCT04342156
37	University of Utah COVID-19 Hydrochloroquine Trial	Drug: Hydroxychloroquine Drug: Placebo oral tablet	https://ClinicalTrials.gov/show/NCT04342169
38	Outcomes Related to COVID-19 Treated With Hydroxychloroquine Among In-patients With Symptomatic Disease	Drug: Hydroxychloroquine Drug: Placebo	https://ClinicalTrials.gov/show/NCT04332991
39	Chloroquine Outpatient Treatment Evaluation for HIV-Covid-19	Drug: Chloroquine or hydroxychloroquine	https://ClinicalTrials.gov/show/NCT04360759
40	A Randomized Controlled Clinical Trial: Hydroxychloroquine for the Treatment of COVID-19 in Hospitalized Patients	Drug: Hydroxychloroquine	https://ClinicalTrials.gov/show/NCT04345692
41	Trial of Hydroxychloroquine In Covid-19	Drug: Hydroxychloroquine Other: Placebo	https://ClinicalTrials.gov/show/NCT04353271
42	Hydroxychloroquine for COVID-19	Drug: Hydroxychloroquine Sulfate Drug: Placebo	https://ClinicalTrials.gov/show/NCT04342221

Table 1 continued

S. no.	Clinical trial study title	Intervention (drug/s)	Weblink to clinical trial
43	Efficacy and Tolerability of Hydroxychloroquine in Adult Patients With COVID-19	Drug: Hydroxychloroquine Sulfate 200 MG [Plaquenil]	https://ClinicalTrials.gov/show/NCT04384380
<i>Clinical trials of Hydroxychloroquine as per severity of Covid-19</i>			
44	Hydroxychloroquine for the Treatment of Mild COVID-19 Disease	Drug: Hydroxychloroquine Drug: Placebo	https://ClinicalTrials.gov/show/NCT04340544
45	Chloroquine, Hydroxychloroquine or Only Supportive Care in Patients Admitted With Moderate to Severe COVID-19	Drug: Chloroquine Sulfate Drug: Hydroxychloroquine Other: Standard supportive care	https://ClinicalTrials.gov/show/NCT04362332
46	Hydroxychloroquine Treatment for Severe COVID-19 Pulmonary Infection (HYDRA Trial)	Drug: Hydroxychloroquine Drug: Placebo oral tablet	https://ClinicalTrials.gov/show/NCT04315896
47	Hydroxychloroquine for the Treatment of Patients With Mild to Moderate COVID-19 to Prevent Progression to Severe Infection or Death	Drug: Hydroxychloroquine Other: The control group will not receive hydroxychloroquine	https://ClinicalTrials.gov/show/NCT04323631
<i>Clinical trials focusing on dose of Hydroxychloroquine against Covid-19</i>			
48	High-dose Hydroxychloroquine for the Treatment of Ambulatory Patients With Mild COVID-19	Drug: Hydroxychloroquine	https://ClinicalTrials.gov/show/NCT04351620
49	Efficacy of Various Doses of Hydroxychloroquine in Pre-Exposure Prophylaxis for COVID 19	Drug: Hydroxychloroquine Sulfate 200 MG Other: Placebo	https://ClinicalTrials.gov/show/NCT04359537
<i>Clinical trials on effect of Hydroxychloroquine in COVID-19 Positive Pregnant Women</i>			
50	Effect of Hydroxychloroquine in COVID-19 Positive Pregnant Women	Drug: hydroxychloroquine sulfate 200 MG Drug: Placebo oral tablet	https://ClinicalTrials.gov/show/NCT04354441
<i>Clinical trial for Hydroxychloroquine in Elderly positive Covid-19 people of age > 65 years</i>			
51	Test and Treat COVID 65plus +	Drug: Hydroxychloroquine Other: Placebo	https://ClinicalTrials.gov/show/NCT04351516

HCQ Hydroxychloroquine

Asymptomatic household contacts of laboratory confirmed Covid-19 cases.

Summary and Future Directions

Covid-19, caused by SARS-CoV-2, had become a pandemic and public health concern considering health and economy related challenges to combat and contain this disease. Due to sudden emergence of Covid-19, the healthcare system was caught unaware without an effective and safe treatment for this infectious disease. Chloroquine

and hydroxychloroquine both are weak bases that tend to accumulate in acidic compartments such as lysosomes and inflamed (acidic) tissues. By raising the intra-lysosomal pH, hydroxychloroquine impairs the autophagic protein degradation [3].

More studies are required to understand the concentration, distribution and effects of hydroxychloroquine in various tissues, as well as interrelationship between given dose and response generated. Identification of exact molecular targets of hydroxychloroquine within the cell as well as on different microbes is the need of hour. More importantly, efficacy of anti-malarial compounds, other than chloroquine and hydroxychloroquine, should be monitored against SARS-CoV including SARS-CoV-2. Newer compounds closely mimicking the hydroxychloroquine like hydroxyferrochloroquine have been synthesized in past, and have shown to exert antiviral effects with some selectivity toward SARS-CoV infection [25]. This approach can be further exploited and/or reinforced to make newer compound with less toxicity and greater selectivity towards SARS-CoV-2.

There are reports of high risk of thrombosis in patients with severe SARS-CoV-2 infection [66] and procoagulant pattern of patients with COVID-19 ARDS justify the clinical reports of thromboembolic complications (pulmonary embolism) during the course of the Covid-19 [67]. Interestingly, hydroxychloroquine has been associated with reduced risk of thrombosis in several in vitro and animal studies. Potential mechanisms by which hydroxychloroquine and chloroquine reduce the procoagulatory state in autoinflammatory diseases include inhibition of antiphospholipid antibody binding [48] or inhibition of platelet aggregation [46, 47, 68]. Notably, in a mouse model of antiphospholipid syndrome, hydroxychloroquine treatment was associated with improvement in endothelial function [69, 70]. The exact molecular mechanisms by which DMARD hydroxychloroquine drug mediate their antithrombotic effects remain largely unknown and lay the platform for future prospective studies.

Acknowledgements Authors duly acknowledge the CDC, NCBI, NIH and WHO for providing free online clinical trials/research articles/information pertaining to Covid-19.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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