Chloroquine and Hydroxychloroquine for the Prevention and Treatment of COVID-19: A Fiction, Hope or Hype? An Updated Review

Sultan AM Saghir,[1] Naif A AlGabri,[2,3] Mahmoud M Alagawany,[4] Youssef A Attia,[5,6] Salem R Alyileili,[7] Shaaban S Elnesr,[8] Manal E Shafi,[9] Omar YA Al-shargi,[10] Nader Al-balagi,[11] Abdullah S Alwajeeh,[12] Omar SA Alsalahi,[13] Amlan K Patra,[14] Asmaa F Khafaga,[15] Ahmed Negida, [16,17] Ahmed Noreldin,[18] Wesam Al-Amarat, [19] Amer A Almaiman,[20] Khaled A El-Tarabily [21,22] Mohamed E Abd El-Hack [4]

[1]Department of Medical Analysis, Princess Aisha Bint Al-Hussein College of Nursing and Medical Sciences, Al-Hussein Bin Talal University, Ma'an, 71111, Jordan; [2]Pathology Department, Faculty of Veterinary Medicine, Thamar University, Dhamar, Yemen; [3]Laboratory of Regional Djibouti Livestock Quarantine, Abu Yasar International Est. 1999, Djibouti, Djibouti; [4]Department of Poultry, Faculty of Agriculture, Zagazig University, Zagazig, 44511, Egypt; [5]Department of Agriculture, Faculty of Environmental Sciences, King Abdulaziz University, Jeddah, 21589, Kingdom of Saudi Arabia; [6]Department of Animal and Poultry Production, Faculty of Agriculture, Damanhour University, Damanhour, Egypt; [7]Department of Integrative Agriculture, College of Food and Agriculture, United Arab Emirates University, Al-Ain, 15551, United Arab Emirates; [8]Department of Poultry Production, Faculty of Agriculture, Fayoum University, Fayoum, 63514, Egypt; [9]Department of Biological Sciences, Zoology, King Abdulaziz University, Jeddah, 21589, Kingdom of Saudi Arabia; [10]Department of Pharmacology, College of Pharmacy, Riyadh Elm University, Riyadh, Kingdom of Saudi Arabia; [11]Ministry of Health, Riyadh, Kingdom of Saudi Arabia; [12]Anti-DopingLab, Doha, Qatar; [13]Department of Medical Laboratories, Faculty of Medicine and Health Sciences, Hodeidah University, Al Hodaidah, Yemen; [14]Department of Animal Nutrition, West Bengal University of Animal and Fishery Sciences, Belgachia, Kolkata, India; [15]Department of Pathology, Faculty of Veterinary Medicine, Alexandria University, Edfina, 22758, Egypt; [16]School of Pharmacy and Biomedical Sciences, University of Portsmouth, Portsmouth, UK; [17]Zagazig University Hospitals, Faculty of Medicine, Zagazig University, Zagazig, Egypt; [18]Histology and Cytology Department, Faculty of Veterinary Medicine, Damanhour University, Damanhour, 22511, Egypt; [19]Department of Medical Support, Al-Karak University College, Al-Balqa' Applied University, Salt, Jordan; [20]Department of Applied Medical Sciences, Community College of Uniazah, Qassim University, Buraydah, 51431, Kingdom of Saudi Arabia; [21]Department of Biology, College of Science, United Arab Emirates University, Al-Ain, 15551, United Arab Emirates; [22]Biosecurity and One Health Research Centre, Harry Butler Institute, Murdoch University, Murdoch, Western Australia, 6150, Australia

Correspondence: Youssef A Attia; Khaled A El-Tarabily
Email yaattia@kau.edu.sa; ktarabily@uaeu.ac.ae

Abstract: In December 2019, the novel coronavirus disease pandemic (COVID-19) that began in China had infected so far more than 109,217,366 million individuals worldwide and accounted for more than 2,413,912 fatalities. With the dawn of this novel coronavirus (SARS-CoV-2), there was a requirement to select potential therapies that might effectively kill the virus, accelerate the recovery, or decrease the case fatality rate. Besides the currently available antiviral medications for human immunodeficiency virus (HIV) and hepatitis C virus (HCV), the chloroquine/hydroxychloroquine (CQ/HCQ) regimen with or without azithromycin has been repurposed in China and was recommended by the National Health Commission, China in mid-February 2020. By this time, the selection of this regimen was based on its efficacy against the previous SARS-CoV-1 virus and its potential to inhibit viral replication of the SARS-CoV-2 in vitro. There was a shortage of robust clinical proof about the effectiveness of this regimen against the novel SARS-CoV-2. Therefore, extensive research effort has been made by several researchers worldwide to investigate whether this regimen is safe and effective for the management of COVID-19. In this review, we provided a comprehensive overview of the CQ/HCQ regimen, summarizing data from in vitro studies and clinical trials for the protection against or the treatment of SARS-CoV-2. Despite the initial promising results from the in vitro studies and the widespread use of CQ/HCQ in clinical settings during the 1st wave of COVID-19, current data from well-designed randomized controlled trials showed no evidence of benefit from CQ/HCQ supplementation for the treatment or prophylaxis against SARS-CoV-2 infection. Particularly, the two largest randomized controlled trials to date (RECOVERY and WHO SOLIDARITY trials), both confirmed that CQ/HCQ regimen does not provide any clinical benefit for COVID-19 patients. Therefore, we do not recommend the use of this regimen in COVID-19 patients outside the context of clinical trials.

Keywords: antiviral drugs, chloroquine, COVID-19, drug safety, hydroxychloroquine, SARS-CoV-2, treatments

Introduction

Coronavirus disease-2019 (COVID-19) is a disease pandemic caused by a new strain of coronavirus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1] Formerly, this disease was referred to as '2019 novel coronavirus' or "2019-nCoV." The virus name (SARS-CoV-2) was chosen because the virus is genetically related to the coronavirus responsible for the SARS outbreak of 2003.[1] While related, the two viruses are different.[1] The spread of SARS-CoV-2 began in Wuhan, China, by the end of December 2019.[2] As of February 17, 2020, the

COVID-19 pandemic has swept the world and infected more than 109,217,366 million individuals worldwide and accounted for more than 2,413,912 fatalities.[2]

The initial case fatality rate of this virus was estimated to be 2% but ranged in some countries from 4 to 9%. After adjustment for asymptomatic cases, this virus's actual fatality rate was estimated to be around 1%. The major challenge of COVID-19 is the rapid transmission of the virus and the substantial proportion of asymptomatic individuals who accounted for 40-50% of transmission.[3]

Extensive efforts are being made to fight this virus, including both pharmacological and non-pharmacological interventions. In the search for potential pharmacologic agents that might be useful to protect against the virus and/or treat COVID-19 patients, clinicians have repositioned chloroquine (CQ) and hydroxychloroquine (HCQ) as a treatment regimen.[3] The rationale for selecting this regimen in the early months of the pandemic was the following: (1) This regimen has been previously utilized for the cure against SARS-CoV-1 with documented success, and (2) recent in vitro experiments in China showed that these agents could inhibit viral replication in vitro.[3]

Since then, this regimen has divided the world with one extreme trolling it as "game changer in medicine" while other touting it as 'useless and dangerous'. Therefore, in the present article, we provide a comprehensive review of the use of CQ/HCQ regimen with or without azithromycin, illustrating the structure, mechanism of action, side effects and drug interactions, and experimental studies data, and data of clinical trials.

Structure of the SARS-CoV-2 Virus

Coronaviruses are spherical with an average diameter of 80-120 nm. They possess a number of club-shaped (17-20 nm) glycoproteins spikes projecting from the surface of the viral envelope.[4] The virus particle contains five major structural proteins, which are glycoprotein spikes (S), an envelope protein (E), matrix protein (M), and nucleocapsid (N) protein.[4] The glycoprotein spikes mediate virus's attachment to different host cell receptors, depending upon the receptor-binding domain (RBD). On attachment to the host cell receptor, the glycoprotein spikes S protein cleavages into two subunits, namely, N-terminal S1 and C-terminal S2 subunit regions by the host proteases enzyme.[4] S1 subunit contains a signal peptide and a RBD. Meanwhile, S2 subunit contains conserved fusion peptide (FP), heptad repeat (HR) peptides, transmembrane domain (TM), and a cytoplasmic domain.[4]

The S1 subunit of SARS-CoV-2 showed 70% identity to Beta coronavirus's S1 subunits (SARS-CoV-1) isolated from human and bats.[5] Human angiotensin-converting enzyme 2 (hACE2) acts as the key receptor to infect the human cells.[5] The S2 subunit plays an important role in mediating the virus fusion and entry into the host cell, in which heptad repeat 1 and 2 (HR1, HR2) can interact with six helical bundles, thereby bringing the viral and cellular membrane in close proximity for fusion.[5]

The ACE2-binding affinity of RBD in S1 subunit of SARS-CoV-2 is 10 to 20-fold higher, which might contribute to the higher infectivity and transmissibility of SARS-CoV-2 compared to SARS-CoV-1.[5] The M glycoprotein is pre–glycosylated M polypeptides with a size range of 25–30 kDa (221–262 amino acids) and gives shape to the virus envelope.[5] Envelope protein (E) is a small polypeptide with a size range of 8.4–12 kDa (76-109 amino acids) and is the integral membrane protein.[4,5]

Chemical Compositions and Sources of CQ and HCQ

CQ and HCQ have similar chemical structures and cellular mechanisms of action.[3] CQ is administered as a phosphate salt, whereas HCQ is administered as a sulfate. Both drugs are absorbed in the upper intestinal tract.[6] The CQ is produced by systematic modification of quinine, which is a plant alkaloid and quinoline containing compound.[7] Hans Andersag discovered CQ in 1934 at the Bayer laboratory and named it "Resochin". It became available in clinical practice in 1947 and quickly became the drug of choice for the treatment of malaria.[7]

CQ, 7-chloro-4-(4-diethylamino-1-methylbutylamino)-quinoline is made by reacting 4-diethylamino-1-methylbutylamine with 4, 7-dichloroquinoline at 180°C.[8] Each of the two components involved in CQ synthesis can be prepared in several ways (Figure 1). In 1946, HCQ sulfate was synthesized as a derivative of CQ by incorporating a hydroxyl group into CQ, and they both share comparable mechanisms of action as weak bases and immuno-modulators.[3]

It was proved that CQ is two to three times as toxic in animals as HCQ.[9] More interestingly, HCQ, compared with CQ, is vastly available to cure auto-immune diseases like rheumatoid arthritis and systemic lupus erythematosus.[10]

Mechanism of Action of CQ and HCQ

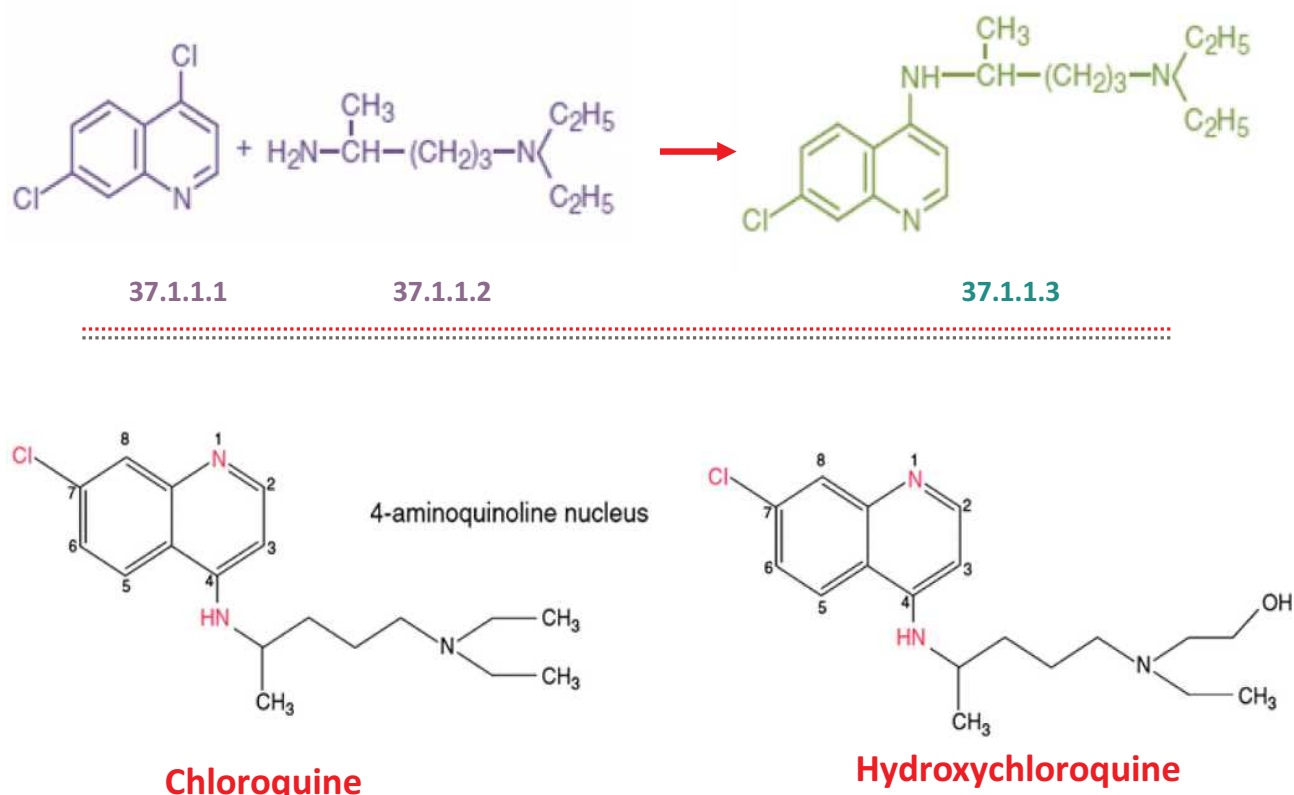Both CQ and HCQ are weak bases that increase the pH of acidic intracellular organelles like lysosomes/endosomes

Figure 1 Chemical composition of chloroquine and hydroxychloroquine.

that require low pH for maturation and function.[11] CQ showed elevation in pH of lysosomes from nearly 4.5 to 6.5 at 100 μM. However, the effect of HCQ on pH values of lysosomes/endosomes is not known due to the lack of studies in this regard.[3]

Moreover, CQ was found to cause changes in the glycosylation of ACE2 spike protein and receptor that ultimately inhibits the entry step and the post-entry phase of SARS-CoV-2.[12] HCQ in the time-of-addition experiment showed its ability to exert the same mechanism (Figure 2).

In addition to the previously known mechanism, a novel mechanism of action for CQ and HCQ on COVID-19 was discovered in 2020 by Fantini et al[13] as it is known that SARS-CoV-2 starts its replication by attaching to the spike (S) viral protein of respiratory cells.[13] The S protein utilizes sialic acids and ACE-2 receptor connected to host cell surface gangliosides for entry. The study showed that CQ (or its more active derivative, HCQ) has a high affinity for binding to gangliosides and sialic acids.[13]

The study also distinguished a novel ganglioside-binding domain (111–158) at the tip of the N-terminal domain of the SARS-CoV-2 S protein. It is expected that this domain can ease attachment with the ACE-2 receptor and enhance contact of the virus to lipid rafts.[13]

Side Effects of the CQ/HCQ Treatment

High doses of CQ were found to cause severe side effects, but it was reported that CQ in a prescribed dose exerts relatively few adverse effects.[14] Ocular adverse effects such as long and subtle symptoms of reduced visual acuity, diplopia, retinal toxicity, and bilateral loss of vision were found to be the most severe side effects caused by high doses of CQ.[15] A high dosage of CQ also causes critical psychiatric issues such as hallucinations, paranoia, and suicidal ideations.[16] Injecting CQ intramuscularly has shown to cause potentially life-threatening hypotension.[17]

Other adverse effects include pruritus, photosensitivity, seizures, paranoia, hallucinations, and retinopathy characterized by the inability to focus on near and far objects[18] (Figure 3). HCQ has a more solubility and less toxic metabolites compared with CQ. Hence, it has fewer adverse effects and is relatively safer.[19] For these reasons, HCQ is often preferred over CQ where possible.[18]
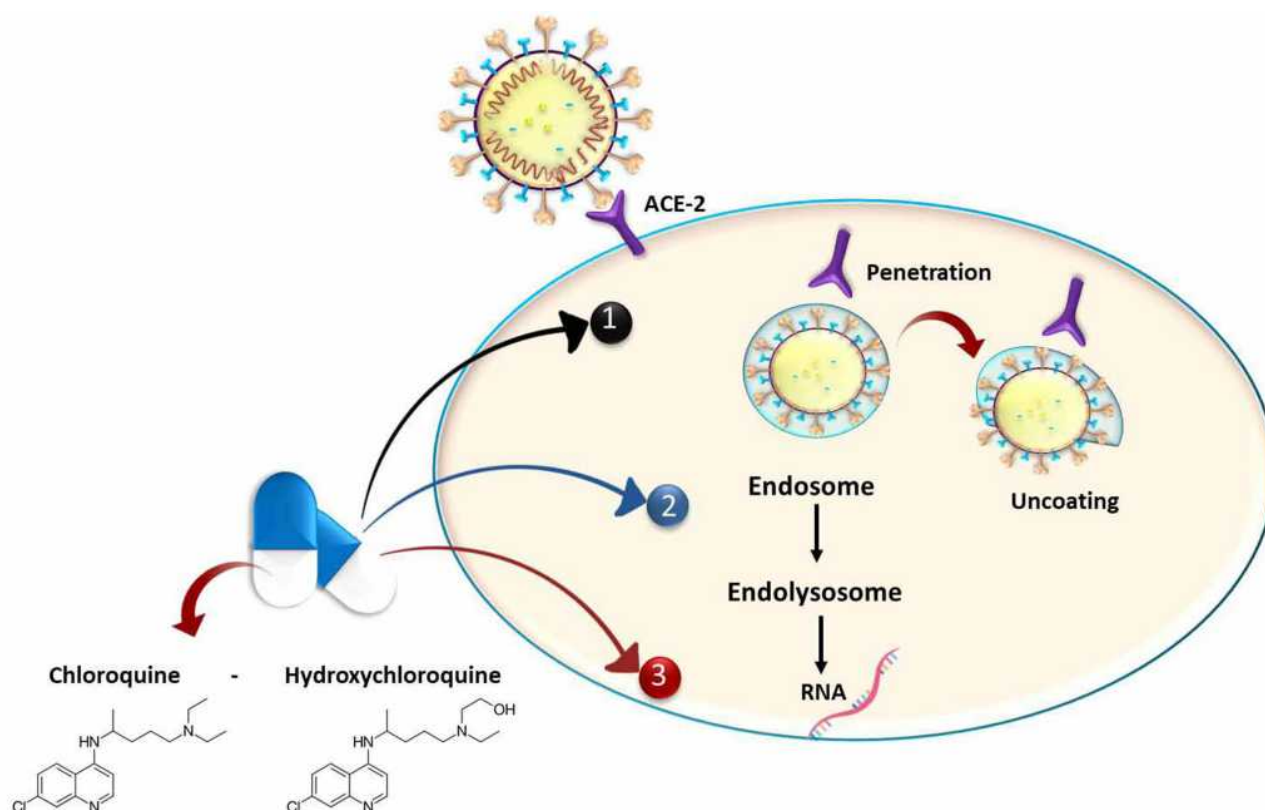
Figure 2 The possible mode of action of chloroquine and hydroxychloroquine versus SARS-CoV-2 infection: (1) interference with the terminal glycosylation of cellular receptor angiotensin-converting enzyme 2 (ACE-2) leads to obstructing virus-receptor attachment; (2) increasing the pH of acidic cellular organelles lead to prevention of endocytosis with adverse influences on post-translational modification of recently synthesized viral RNA and virion transport; (3) blocking of viral protein synthesis and virion assembly.

Cautions and Contraindications

Patients receiving CQ or/and HCQ must be monitored for their haematological parameters (RBC, WBC, and platelet counts), blood glucose (hypoglycemic risk of HCQ), serum electrolytes, renal as well as hepatic functions.[20] Electrocardiography (ECG) is essential before starting therapy with these medications and the concomitant use of these drugs with other drugs known to extend the corrected QT (QTc) interval of the heart (like antihistamines, anti-depressants, anti-arrhythmic, anti-psychotics, moxifloxacin, teneligliptin, and ondan-setron) should be averted.[21] The addendum of HCQ to azithromycin, as reported by Gautret et al[22] in the French trial, may elevate QTc extension.[23] If QTc is 450–500 msec, it is recommended to do daily ECG. CQ and HCQ must not be utilized simultaneously with ritonavir/lopinavir and remdisivir for expected QTc extension. Additionally, hypoglycaemia should be observed in diabetes patients, particularly with conco-mitant usage of CQ/HCQ and ritonavir/lopinavir.[23]

Pharmacovigilance on the mental and visual disorder is also carefully wanted (Figure 4).

Despite case reports of reversible heart failure and CQ-induced cardiomyopathy in the literature, large meta-analysis and numerous investigations carried out in patients having rheumatoid arthritis confirmed a lowered cardiovascular hazard with both drugs; nonetheless, a baseline ECG must be completed in patients with certain cardiovascular disease.[24] Every clinician utilizing these drugs should realize contraindications to both compounds; porphyria, pre-existing maculopathy, retinopathy, glucose-6-phosphate dehydrogenase deficiency, epilepsy, recent myocardial infarction, hypersensitivity to these agents, and QTc>500 msec.[20] There is no evidence that CQ and HCQ are contraindicated in lactating and pregnant women.[25]

It is worth noticing that CQ and HCQ interact with various drugs; many lead to QT prolongation and might lead to serious cardiac events and death. As mentioned earlier, this includes patients who take the CQ/HCQ
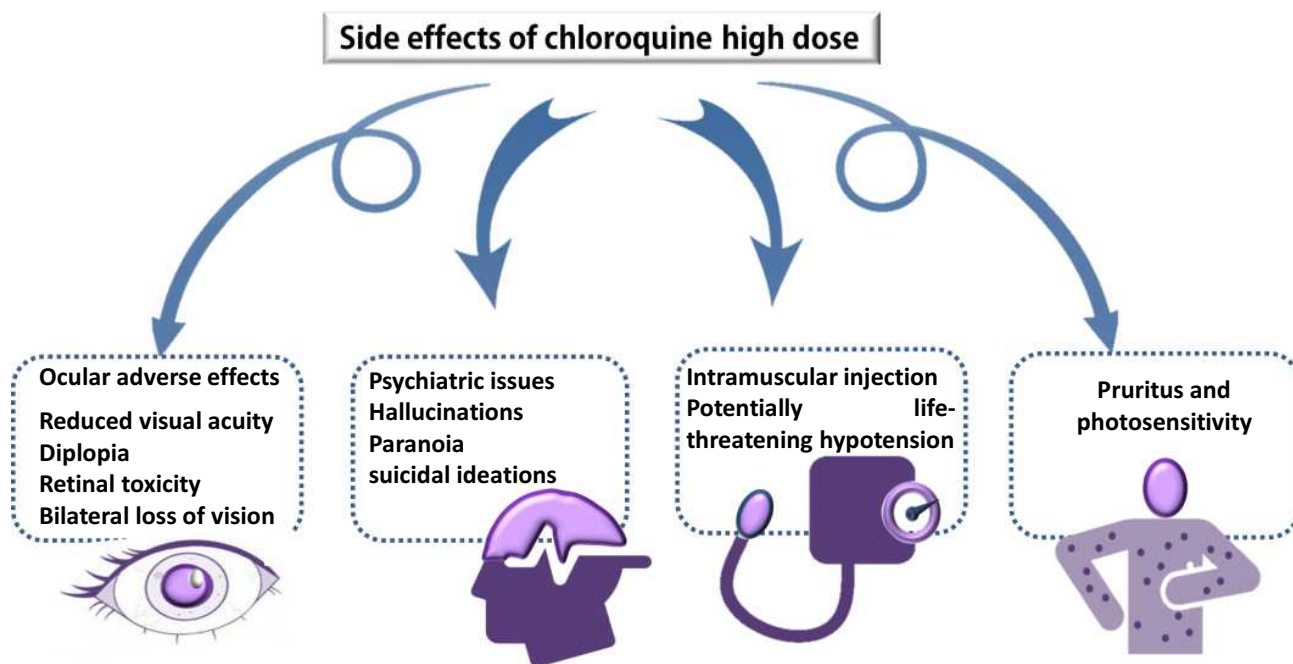
Side effects of chloroquine high dose

Ocular adverse effects

Reduced visual acuity
Diplopia
Retinal toxicity
Bilateral loss of vision

Psychiatric issues
Hallucinations
Paranoia
suicidal ideations

Intramuscular injection
Potentially life-threatening hypotension

Pruritus and photosensitivity

Figure 3 The possible side effects of chloroquine and hydroxychloroquine.

Cautions and contraindications

CQ and HCQ are contraindicated in patients suffered:

• Porphyria
• Pre-existing maculopathy
• Retinopathy
• G6pd deficiency
• Epilepsy
• Recent myocardial infarction
• Hypersensitivity to these agents

Avoided the concomitant use of some drugs such as:
• Anti-histamines
• Anti-depressants
• Anti-arrhythmic
• Anti-psychotics
• Moxifloxacin
• Teneligliptin
• Ondansetron
• Azithromycin
• Lopinavir/Ritonavir
• Remdisivir

Patients must be monitored for:

• Hematological parameters (RBC, WBC, and platelet counts)
• Blood glucose (hypoglycemic risk of HCQ)
• Serum electrolytes
• Renal and hepatic functions

ECG is essential before starting therapy and daily ECG if QTc is 450–500 msec.

Pharmacovigilance on visual and mental disturbance is closely required.

There is no evidence that CQ HCQ are contraindicated in lactating and pregnant women

Reversible heart failure cardiomyopathy was noted in some patients (baseline ECG should be done in patients with established cardiovascular disease)

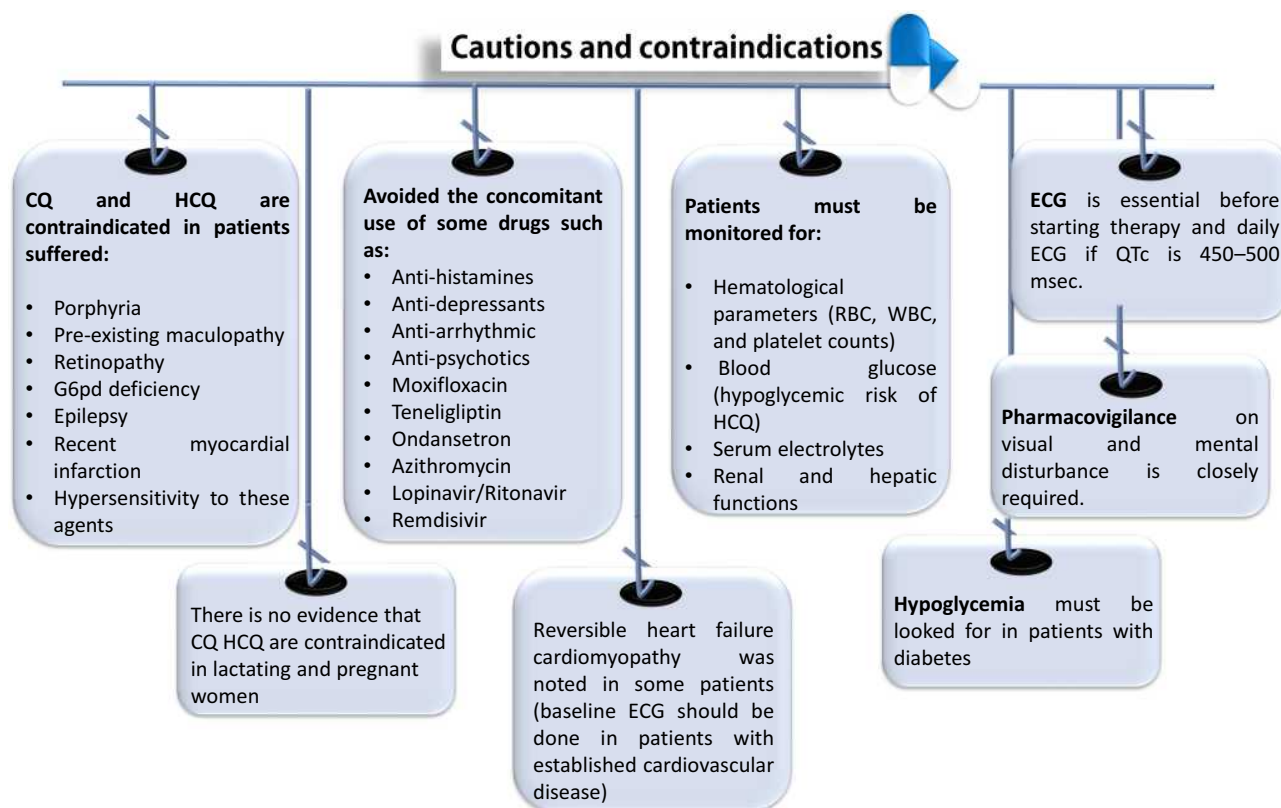**Hypoglycemia** must be looked for in patients with diabetes

Figure 4 Cautions and contraindications during treatment with chloroquine (CQ) and hydroxychloroquine (HCQ).

regimen with azithromycin. Such patients require close cardiac monitoring as long as they are on the CQ/HCQ regimen.[23] Besides, CQ/HCQ might decrease blood glucose; therefore, these drugs can be used with caution in patients with diabetes mellitus. A recent study showed that using these drugs during the pandemic contributed to

Table I The Commonest Drug Interactions and Disease Interactions of the Chloroquine (CQ) and Hydroxychloroquine (HCQ) Regimen

	CQ	HCQ
Drug interactions	• Hydroxyzine • Azithromycin • Ciprofloxacin • Duloxetine • HCQ • Levetiracetam • Pregabalin • Mefloquine • Primaquine • Albuterol	• Amitriptyline • Calcium/Vitamin D • Duloxetine • Leflunomide • Albuterol • Tramadol
Disease interactions	• Oculotoxicity • Porphyria • Arrhythmias • Bone marrow suppression • Ototoxicity • Seizures • Glucose-6-PD deficiency • Hepatotoxicity • Myasthenia gravis • Psoriasis	• Oculotoxicity • Porphyria • Arrhythmias • Bone marrow suppression • Ototoxicity • Seizures • Glucose-6-PD deficiency • Hepatotoxicity • Myasthenia gravis • Psoriasis • Diabetes • Heart disease • Renal impairment

hypoglycaemic events.[26] A summary of the common drug and disease interactions of CQ and HCQ are shown in (Table 1).

Methods of Selecting Studies for This Review

We searched PubMed, SCOPUS, and Web of Science until December 31, 2020, using the keywords "(chloroquine OR hydroxychloroquine) and (COVID-19 OR SARS-CoV-2 OR 2019-nCOV)". Studies were screened for eligibility for this review. Studies meeting the following conditions were reviewed (1) study design: experimental animal studies and prospective clinical trials, (2) study drug: chloroquine and hydroxychloroquine, (3) outcomes: viral inhibition in experimental studies and mortality or time to recovery in clinical trials. Studies that do not satisfy these criteria were excluded from the review. Eligible studies were presented in tables and narratively discussed in the text.

Experimental Studies

The continuous and rapid spread of the COVID-19 pandemic has led to extensive ongoing efforts worldwide to develop effective and safe therapy. CQ and HCQ in COVID-19 are among the drugs being tested, which were reported on February 4, 2020, to suppress SARS-CoV-2 in vitro. There is considerable in vitro evidence that CQ and HCQ are efficient in preventing SARS-CoV-2 vigour. Liu et al[3] detected that both drugs have a 50% cytotoxic concentration (CC50). However, the 50% maximum efficient concentration was lower for CQ than HCQ (EC50 – the dose at which viral RNA elevation is suppressed by 50%) regardless of the multiplicity of infection (MOI – the ratio of virions to host cells).[3]

Wang et al[27] found that CQ has in vitro antiviral vigour with an EC50 of 1.13 µM and CC50 >100 µM at an MOI of 0.05 and shown that the eclecticism for SARS-CoV-2 is high compared with that for host cells. The study also showed that CQ at a concentration of 0.36 mg/L decreased viral load by 50% in vitro using Vero E6 cells.[27]

Yao et al[28] also proved the activity of CQ versus SARS-CoV-2 and detected that CQ was less potent than HCQ in vitro versus SARS-CoV-2 (EC50 of 5.47 µM and 0.72 µM, respectively, MOI = 0.01). Based on physiologically based pharmacokinetic (PBPK) model results, oral HCQ sulfate with a supplying dose of 400 mg twice a day then 200 mg twice a day as a maintenance dose for four days is advised for SARS-CoV-2 infection, and it is three times more potent than CQ phosphate when given 500 mg twice per day for five days in advance.[28]

Clinical Trials on CQ/HCQ Regimen for the Protection Against SARS-CoV-2 Infection

Although preclinical evidence suggests that CQ and HCQ can inhibit viral replication and might prevent COVID-19, the current evidence does not support their prophylaxis efficacy against SARS-CoV-2 infection.[27]

Expert opinions advised using the CQ/HCQ regimen for prophylaxis against SARS-CoV-2 infection, particularly between healthcare laborers who are at higher hazard of infection.[29,30] However, this opinion was refuted by data from a well-designed randomized controlled trial on 821 participants. Participants were allocated to be administrated with either HCQ or placebo within four days after exposure. The happening of novel symptoms compatible with COVID-19 did not vary markedly among the two groups (11.8% versus 14.3%; P=0.35).[31]

Clinical Experiments on CQ/HCQ Regimen for the Therapy of COVID-19

Recent literature has suggested that CQ/HCQ drugs could be used as antiviral drugs to cure COVID-19 infections.[24] In addition, Iyer et al[32] stipulated that the CQ can block the quinone reductase-2, a fundamental agent needed for the sialic acid biosynthesis that SARS-CoV-2 utilizes it as the receptor moieties. A recent small clinical study by Gautret et al[22] reported that positive SARS-CoV-2 in nasopharyngeal secretions significantly decreased on day six after inclusion in HCQ-treated COVID-19 patients against patients who received supportive care only.[22] The CQ elevates pH in host cell lysosomes and passively affects virus–receptor linking and intervenes with the glycosylation of SARS-CoV-2 receptors. Additionally, it showed a hopeful antiviral influence versus SARS-CoV-2 in vitro and limited the course of the disease and enhanced COVID-19-pneumonia patients.[33]

The first evidence of CQ effectiveness in COVID-19 came from China in February 2020 by the Chinese government.[34] These data reported that CQ phosphate was given to over 100 patients in China and reduced the duration of illness and significantly improved pneumonia infection and lung imaging. There were no adverse events reported. It seems that combining data from various in-progress trials using a variety of study designs released such findings. A study by Gautret et al[22] in France on March 17, 2020, considered as the first clinical trial, was conducted as an open-label non-randomized controlled experiment.[22] The trial included patients who suffered from SARS-CoV-2 among which 22 of the 36 patients included in the study had symptoms in the upper respiratory tract, eight had symptoms in the lower respiratory tract, while six patients were asymptomatic.[22] The experimental group (22 patients) was treated with HCQ 200 mg three times per day for ten days, whereas the control group was treated with ordinary care.[22] Azithromycin was also prescribed for six patients of the treatment group to prevent bacterial superinfection. In this trial, SARS-CoV-2 carriage at day 6 was the primary outcome which was examined by testing nasopharyngeal swabs utilizing PCR of SARS-CoV-2 RNA.[22]

The experiment's outcomes revealed that the experimental group was markedly tested negative for the virus than patients in the control group (70% vs. 12.5% virologically cured, $P<0.001$) on day 6. Furthermore, the results of HCQ and azithromycin combination were astonishing as all patients treated with this combination were negative on day 6. The study proved the efficiency of HCQ and the possible synergistic influence of its combination with azithromycin needs further declaration, as suggested by Gautret et al.[22]

Despite this trial's favourable outcomes, severe limitations have made its results questionable.[35] First, there was recruitment for an additional six patients but were excluded, and no intention-to-treat analysis was performed due to many reasons that have led to the failure of following-up these patients.[35,36] Secondly, the researchers added that the sample size was not enough to achieve 85% power, which required recruiting 48 patients for the required power to be achieved. The overstatement of influence sizes and false-positive outcomes can be expected from the underpowered trial with a sample size of 36 patients.[37] On the sixth day, the researchers reported that a patient showed negative for the virus but revealed positive on the eighth day, which raised a concern about a trial lacking for long-term and medium follow-up data since the primary outcome is viral PCR status at day 6.[37] This incidence indicates that long-term data of CQ/HCQ effectiveness in the therapy of COVID-19 is necessary. Finally, the trial's allocation bias cannot be denied where there was no randomization for patients to the control and treatment groups.[37]

Another pilot study published on March 25, 2020, by Chen et al[38] who evaluated the safety and efficacy of HCQ in the management of patients with COVID-19. A sum of 30 patients diagnosed with COVID-19 was recruited and randomly allocated (1:1) into the treatment and control groups. The test group treated with oral CQ sulfate (400 mg one time a day for five days) based on conventional treatment, while the control group received traditional treatment.[38] The principal outcome was the negative change rate of COVID-19 nucleic acid in respiratory pharyngeal swab on the seventh day. On day 7, the test group's throat swabs showed negative COVID-19 nucleic acid in 13 patients (86.7%), with one case progressed to severe during the treatment.[38] In comparison to the treatment group, 14 (93.3%) subjects in the control group ($P>0.05$) also tested negative. The average period between virus nucleic acid negative maintenance and patients' hospitalization in the test and control groups was 4 (1–9) days and 2 (1-4) days, respectively (U=83.5, $P>0.05$).[38] In terms of safety, abnormal liver function and transient diarrhea in the experimental and the control groups subjects were noticed in 4 (26.7%) and 3 (20%) cases, respectively ($P>0.05$).

The small sample size in this study has made a general conclusion that the prediction of typical COVID-19 patients is perfect.[38]

Following that, an extensive argument was raised against Gautret et al[22] study by Kim et al.[39] It was reported that there was a rush in judgment of the study due to the pressing requirement for efficient therapy for SARS-CoV-2. The clinical trial's limitations were discussed, such as using an invalidated replacement endpoint, deficiency of blinding or randomization, and including the small sample size. Another study highlighted methodological flaws that were considered to impact the validity of the findings.[40]

Despite the limitations in the first clinical trial, its promising results ended up advising the usage of CQ/HCQ in the management of COVID-19 officially by guidelines. The National Health Commission published the recommendation of treatment COVID-19 by CQ, China, published in mid-February 2020, indicating that 500 mg CQ phosphate (equivalent to 300 mg CQ) twice per day for ten days is recommended for patients with COVID-19.[41] On March 17, 2020, other recommendations published by the L. Spallanzani National Institute for Infectious Disease in Italy, in which the combination of CQ (500 mg CQ per day) or HCQ (200-500 mg HCQ per day) with a different antiviral drug is indicated for COVID-19.[42]

A pharmacokinetic study in France aimed to optimize HCQ dosing in the intensive care unit (ICU) of COVID-19 patients was carried out by Perinel et al.[43] The study recruited 13 patients in ICU who were treated by HCQ at a dose of 200 mg twice per day. The mean age of patients was 68 years, 31% with moderate or severe renal failure, and 46% were obese.[43] The study demonstrated that the dosing regimen of 200 mg thrice a day is inappropriate to reach a supposed target blood level of 1–2 mg/L in this population. According to data from patients with rheumatoid arthritis and the 161 blood levels registered, the proposed dosing regimen delivers a dose of 800 mg once per day on the first day, then 200 mg twice per day for seven days.[43]

The efficacy of combining azithromycin and HCQ was also evaluated by an uncontrolled non-comparative observational study carried out by Gautret et al[22] in 80 patients diagnosed with a relatively mild infection of COVID-19. Six days were set as the minimum follow-up period. There was a clinically marked amelioration in all patients, except for one patient aged 86 years who died, and another patient (74-year-old) was still in the ICU. The viral load

of nasopharyngeal samples rapidly decreased. Of these samples, 83% of the patients were tested negative on the seventh day, while on the eight's day, 93% were negative.[22] On day 5 of the treatment, respiratory samples' viral cultures were found negative in 97.5% of the patients.[22] Therefore, patients were quickly got out of the infectious disease unit with five days as an average length of stay. Although the number of patients was just 80 and the severity of the illness was mild, the study reflected an excellent picture of the combination of azithromycin and HCQ.[22]

Regarding the optimal dose of HCQ in COVID-19 patients, Garcia-Cremades et al[44] tested the safe and effective dosage of HCQ for COVID-19 treatment. It was predicted that doses of over 400 mg twice a day of HCQ for ≥5 days reduced viral loads quickly, shortening the treatment course, decreasing the number of patients with detectable SARS-CoV-2 infection.[44] In contrast, increasing the dose of HCQ to over 600 mg twice a day has more probability of prolonging QTc intervals.[44] In recent study from Belgium, Catteau et al[45] have shown that the low dose HCQ monotherapy has reduced mortality rate compared with the non-HCQ treated patients.[45]

A study from South Korea by Lee et al[46] investigated the effectiveness of post-exposure prophylaxis after a significant exposure of COVID-19 in a long-term care hospital using HCQ (400 mg orally daily till the end of 14 days of quarantine) in 211 persons containing 22 health-care workers and 189 patients, with negative PCR checks for COVID-19.[46] After completing the post-exposure prophylaxis period by 184 patients and 21 care-workers without any severe effects, all PCR tests were negative at the ending of the 14 days of quarantine.[46] The shortage of control groups in the study and having other 29 hospital staff who tested negative after the 14 days of quarantine although they did not receive post-exposure prophylaxis (Although being classified low-risk exposure) are considered essential limitations in the study.[46] In a study highlighted COVID-19 and immunomodulation in inflammatory bowel diseases (IBD), Neurath[47] mentioned that there is a possibility for drug–drug interactions between HCQ or IBD therapies. The risk of interaction is potentially increased by combination of medication with HCQ and infliximab/adalimumab for nerve harm.[47]

However, there is no evidence to discontinue IBD-specific medications in COVID-19 patients cured with such drugs. The favourable effect of HCQ and azithromycin combination on the clinical results and viral loads of

patients infected with COVID-19 has led to implementing the regimen by clinicians worldwide.[48] On the other hand, both drugs have been independently revealed to influence the electrical system of the heart, causing QT-interval elongation, drug-induced torsades de pointes, and drug-stimulated sudden cardiac death.[48]

In this context, an American study carried out by Chorin et al[49] examined the QT-interval in 84 patients with COVID-19 cured with a combination of HCQ (400 mg daily on day one, then 200 mg daily from day 2 to 5) and azithromycin (500 mg per day for five days). After 4.3 ± 1.7 days as an average time for exposure to HCQ/azithromycin, ECG was followed up.[49] It was found that the QTc markedly extended. In a group of nine (11%) of those patients, there was a severe prolongation of the QTc to >500 ms, which is a marker of a high danger of sudden cardiac death caused by malignant arrhythmia.[49] Out of the group of nine patients, five patients had a normal QTc. It was suggested that regular evaluation for QTc must be implemented by patients with COVID-19 who are cured with a combination of HCQ/azithromycin combination, especially those who have comorbidities or/ and with other QT-prolonging medications.[49]

A randomized clinical experiment by Borba et al[50] from Brazil compared the effect of high doses (600 mg twice per day for ten days) against small doses (450 mg twice a day on day one and OD for four days) of CQ diphosphate as adjunctive therapy for 81 adult patients treated with SARS-CoV-2 infection.[50] Forty patients received low doses, while 41 received high doses. In the small dose group, 15.0% (6 out of 40) of patients died on day 13 days compared with 39% of the high-dose group (16 of the 41 patients). Regarding safety, 4 of 36 patients (11.1%) receiving low-dose experienced prolongation of QTc interval compared with 7 of 37 (18.9%) patients receiving the high-dose.[50] Besides, ventricular tachycardia was developed in 2 patients (2.7%) in the high-dose group. As a result of these findings, the trial was stopped. It was inferred that the high dosage of CQ must not be advised for adversely ill patients with COVID-19.[50]

Patients with systemic lupus erythematosus (SLE) were a population of interest for Mathian et al.[51] SARS-CoV-2 represents a source of concern for the management of patients with SLE. In patients with SLE, the use of immunosuppressive drugs, the intrinsic perturbations of the immune response, and the potential presence of organ damage associated with their disease make those patients at higher risk of severe infections. Currently, and as a part

of SLE treatment, HCQ is a standard long-term drug for SLE.[52]

HCQ also has antiviral activity in COVID-19, and its therapeutic or even prophylactic activity for COVID-19 was proved by preliminary clinical trials. Mathian et al[51] examined the clinical observations of COVID-19 in a series of 17 patients with SLE receiving long-term treatment of HCQ (median of 7.5 years) and with obesity and chronic kidney disease as comorbidities.[51] Although this study gave an initial clinical view of the infection course in patients with SLE cured with HCQ, it did not conclude the severity and incidence rate of COVID-19 in SLE. Moreover, it was also shown that HCQ does not protect against COVID-19, at least its negative practice, in patients with SLE.[51]

On the other hand, strong evidence from a well-designed randomized controlled trial (RCT) does not advocate the usage of CQ/HCQ regimens in COVID-19 patients. Data from the UK's recovery trial, the world's largest COVID-19 clinical trial to date, by Horby and Landray[53] showed that HCQ did not reduce the 28-day mortality rate among COVID-19 patients compared to the standard of care.[53] While these outcomes were questioned by several experts owing to the relatively higher loading dose.

On the first day of the study (2400 mg in 24 hours), similar findings were reached by the WHO's solidarity trial in several countries worldwide. On June 5, 2020, the WHO announced that based on an interim analysis of the trial data, HCQ did not reduce the mortality compared to the standard of care.[54] The characteristics of the in vitro studies on SARS-CoV-2 and clinical trials studying the efficacy of CQ and HCQ in COVID-19 patients are illustrated in Table 2.

Past Experiences, Current Situations, and Future Directions

Based on the review of the existing literature, the CQ/ HCQ regimen gained worldwide attention. It showed a promise in the preclinical experiments and some clinical studies during the early months of the pandemic. Nonetheless, the usage of the CQ/HCQ regimen in treating COVID-19 has been challenged by the recent data from well-designed RCTs. The CQ and HCQ are widely used for the first-line of treatment against the malarial parasite in most endemic Asia and African countries.[63] Besides malaria treatment, CQ is utilized in rheumatoid arthritis,

Table 2 Characteristics of the in vitro Investigations on SARS-CoV-2 and Clinical Trials Studying the Efficacy of Chloroquine and Hydroxychloroquine in COVID-19 Patients

Reference and Country	Population (n Patients)	Intervention and Comparison Groups	Primary Outcomes
China[28]	in vitro study with SARS-CoV-2-infected Vero cells	Infected Vero cells were treated with CQ or HCQ at 0.032, 0.16, 0.80, 4, 20, or 100 μM for 24 or 48 h.	• CQ and HCQ decreased viral replication in a concentration-dependent manner. • EC_{50} values for CQ were 23.90 and 5.47 μM at 24 and 48 h, respectively. • EC_{50} values for HCQ were 6.14 and 0.72 μM at 24 and 48 h, respectively.
China[28]	in vitro study with Vero cells	Vero cells were pre-treated CQ or HCQ at 0.032, 0.16, 0.80, 4, 20, or 100 μM for two h and were then infected with SARS-CoV-2 and incubated for 24 or 48 h.	• HCQ showed a higher in vitro antiviral influence in comparison with CQ. • The EC_{50} values for CQ were greater than 100 and 18.01 μM at 24 and 48 h, respectively. • EC_{50} values for HCQ were 6.25 and 5.85 μM at 24 and 48 h, respectively.
China[3]	in vitro study with African green monkey kidney VeroE6 cells	SARS-CoV-2 infected cells at four different multiplicities of infection (MOI) and treated with CQ or HCQ up to 50 μM for 48 h	• CC_{50} values of CQ and HCQ were 273 and 250 μM, respectively, which are not significantly different. • At all MOI (0.01, 0.02, 0.2, and 0.8), EC_{50} for HCQ (4.51, 4.06, 17.31, and 12.96 μM) was higher than that of CQ (2.71, 3.81, 7.14, and 7.36 μM). • Statistically, the variations in EC_{50} values were significant at MOI of 0.01 ($P < 0.05$) and 0.2 ($P < 0.001$).
China[27]	in vitro study with Vero E6 cells.	Cells were infected with SARS-CoV-2 at MOI of 0.05 in the presence of different concentrations of CQ, penciclovir, ribavirin, nafamostat, nitazoxanide, remdesivir, favipiravir and chloroquine.	• EC_{50}, SI index, and CC_{50} values for CQ were 1.13 μM, >100 μM, and 88.5. • These values were higher for for ribavirin (EC_{50} = 110 μM, CC_{50} > 400 μM, and SI > 3.65), penciclovir (EC_{50} = 96.0 μM, CC_{50} > 400 μM, SI > 4.17) and favipiravir (EC_{50} = 61.9 μM, CC_{50} > 400 μM, SI > 6.46), nafamostat (EC_{50} = 22.50 μM, CC_{50} > 100 μM, SI > 4.44), and was comparable to nitazoxanide (EC_{50} = 2.12 μM; CC_{50} > 35.53 μM; SI > 16.76) and remdesivir (EC_{50} = 0.77 μM; CC_{50} > 100 μM; SI > 129.87) for EC_{50}.
France[22]	Age >12 years and positive for SARS-CoV-2. Patients with HCQ or CQ allergy were excluded or had another recognized contraindication to cure with the drug. Pregnant and breastfeeding patients were excluded.	Oral HCQ 200 mg TD × ten days (n=20). Symptomatic treatment and AZT (n = 6; 500 mg/d on day 1 then 250 mg/d for next 4 days) with HCQ. Patients (n=16) who rejected the cure or had relegation criteria, served as controls.	• Control patients were younger than HCQ-treated patients (37.3 years vs 51.2 years). • At sixth day post-inclusion, 70% of HCQ-cured patients were negative compared with 12.5% in the control group (p= 0.001). • At day six post-inclusion, 100% of patients treated with combination of HCQ and AZT were negative compared with 57.1% in patients cured with HCQ only, and 12.5% in the control group (p<0.001).

(Continued)

Table 2 (Continued).

Reference and Country	Population (n Patients)	Intervention and Comparison Groups	Primary Outcomes
China[38]	Confirmed COVID-19 patients. Thirty patients were randomly grouped into treatment and control groups.	Oral HCQ sulfate 400 mg OD × 5 days (n=15). No HCQ was provided to Patients (n=15).	• On day 7, the number of negative samples did not differ (13 (86.7%) cases in the HCQ group versus 14 (93.3%) cases in the control group; $P>0.05$) • The period from hospitalization to negative result of virus nucleic acid did not differ (4 ± 1.9 days in HCQ versus 2 ± 1.4 days in the control group; $P>0.05$). • The time for body temperature normalization was comparable (1 ± 0.2 day 1 HCQ group versus 1 ± 0.3 days in the control group). • Radiological progress was noted on CT images in 7 cases (46.7%) of the control group and 5 cases (33.3%) of the HCQ group, and all patients revealed amelioration in follow-up examinations. • Three cases (20%) of the control group and four cases (26.7%) of the HCQ group had abnormal liver function and transient diarrhoea ($P>0.05$).
South Korea[46]	COVID-19 exposed individuals (211 containing 22 careworkers and 189 patients) with negative PCR tests for COVID-19 in a long-term care hospital in Korea. Four patients and one coworker were not finally completed.	COVID-19 exposed individuals were administered HCQ at 400 mg OD x 14 days during the quarantine. No control groups.	• At the ending of two weeks of quarantine, all follow-up PCR tests were negative. • A sum of 32 individuals (15.6%) mentioned one or more symptoms through post-exposure prophylaxis. • The most common symptoms were skin rash (4.3%), loose stool or diarrhoea (9%), bradycardia (0.95%), and gastrointestinal upset (0.95%). Post-exposure prophylaxis was stopped in 5 patients (2.7%) because of the requirement for fasting (1), bradycardia (2), and gastrointestinal upset (2).
Netherlands[55]	Patients (n = 95) were aged 18 years or older and suspected of having COVID-19 disease.	CQ was a loading dose of 600 mg followed by 300 mg BD (starting 12 h after the loading dose), for the next four days	• CQ treatment in patients with COVID-19 markedly extended the QTc interval by 34–35 ms; 23% of the patients had a QTc interval exceeding 500 ms. Statistically marked influences were detected on QRS interval (mean difference 6 ms), PR interval (mean difference 8 ms), and heart rate (mean difference −10 bpm).

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Table 2 (Continued).

Reference and Country	Population (n Patients)	Intervention and Comparison Groups	Primary Outcomes
Netherlands[23]	A retrospective investigation of 251 patients having COVID-19.	HCQ was orally administrated at 400 mg BD for one day (loading dose) then 200 mg BD for four days. AZT was orally administrated for five days at a dose of 500 mg OD.	• The QTc interval extended from a baseline of 439 ± 29 ms to a maximum value of 473 ± 36 ms ($P < .001$), which happen on day 4.1 ± 2 of treatment. • Extreme novel QTc interval extension to >500 ms revealed in 23% of the patients. • One patient showed polymorphic ventricular tachycardia.
USA[56]	A retrospective investigation of 1376 patients having COVID-19.	HCQ (n = 811) was provided at 600 mg BD on day 1, followed by 400 mg/d for 4 next days. Control group patients were less adversely ill at baseline than those with HCQ-treated patients (n = 565; the ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen, 223 vs 360).	• HCQ use was not accompanied with a markedly lower or higher hazard of death or intubation (hazard ratio, 1.04; 95% CI, 0.82 to 1.32).
USA[57]	A retrospective investigation of 368 patients diagnosed with COVID-19.	HCQ (n = 97) alone and HCQ + AZT (n = 113) in combination. In the control group (n = 158), no HCQ was provided.	• The hazard of death from any reason was elevated in the HCQ group (adjusted hazard ratio, 2.61; 95% CI, 1.10 to 6.17; P=0.03). • The risk of death was similar in the HCQ+AZ group (adjusted hazard ratio, 1.14; 95% CI, 0.56 to 2.32; P=0.72). • The hazard of ventilation was comparable in the HCQ group (adjusted hazard ratio, 1.43; 95% CI, 0.53 to 3.79; P=0.48) and the HC+AZ group (adjusted hazard ratio, 0.43; 95% CI, 0.16 to 1.12; P=0.09).
France[58]	A retrospective investigation of 181 patients having COVID-19 and requiring oxygen ≥ 2 L/min.	HCQ (n = 84) 600 mg/d for 7 day In control (n = 97), no HCQ was provided.	• 20.2% of the patients in the HCQ group were died within seven days or moved to the ICU vs 22.1% in the no–HCQ group (16 vs 21 events, the relative hazard of 0.91, 95% CI 0.47-1.80) in the HCQ group. • The death of 2.8% of the patients was within seven days vs 4.6% in the no–HCQ group (three vs four events, the relative risk of 0.61, 95% CI 0.13–2.89). • 27.4% in the HCQ group versus 24.1% in control group patients developed acute respiratory distress syndrome within seven days (24 vs 23 events, relative risk of 1.14, 95% CI 0.65-2.00). • 8 patients receiving HCQ (9.5%) revealed electrocardiogram modifications requesting HCQ stop.

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Table 2 (Continued).

Reference and Country	Population (n Patients)	Intervention and Comparison Groups	Primary Outcomes
USA[59]	A retrospective investigation of 181 patients having COVID-19.	HCQ at 200–600 mg OD/BD (n = 271) alone; HCQ + AZT (n = 735) in combination; AZT 200–500 mg once/OD/BD (n = 211), and no drug (n =221)	• The death of patients treating with AZT alone, 21/211 (10.0% (95% CI, 5.9%-14.0%)), HCQ + AZT was 189/735 (25.7% (95% CI, 22.3%-28.9%)), HCQ alone, 54/271 (19.9% (95% CI, 15.2%-24.7%)), and neither drug, 28/221 (12.7% (95% CI, 8.3%–17.1%)). • Co marked variations in mortality for patients receiving HCQ + AZT (hazard ratio of 1.35 (95% CI, 0.76–2.40)), HCQ alone (hazard ratio of 1.08 (95% CI, 0.63–1.85)), or AZT alone (hazard ratio of 0.56 (95% CI, 0.26–1.21)) in comparison with patients administrating neither drug. • Cardiac arrest was markedly higher in patients receiving HCQ + AZT (adjusted OR, 2.13 (95% CI, 1.12–4.05)), but not HCQ alone (adjusted OR, 1.91 (95% CI, 0.96–3.81)) or AZT alone (adjusted OR, 0.64 (95% CI, 0.27-1.56)) compared with patients receiving neither drug.
China[60]	A retrospective investigation of 181 patients having COVID–19 and treated with HCQ.	HCQ 400 mg/d (200 mg BD) for 7–10 days (n = 48). In the control group (n = 520), no HCQ was provided.	• Mortalities reduced in HCQ group (18.8% (9/48) versus 45.8% (238/520) in control group (p<0.001)). • The time of hospitalization before patient death was 15 (10–21) days for the HCQ group versus 8 (4–14) days for control groups (p<0.05). • The level of inflammatory cytokine IL–6 markedly decreased from 22.2 (8.3–118.9) pg/mL to 5.2 (3.0-23.4) pg/ml (p<0.05) in the HCQ group, but there is no alteration in the control group.
Recovery trial UK[61]	An ongoing randomized controlled trial of more than 11,000 COVID-19 patients to date	HCQ(200 mg tablet containing 155 mg base equivalent) received a loading dose of four tablets (800 mg) at zero and six hours, then two tablets (400 mg) starting at twelve hours after the initial dose and then every twelve hours for the next nine days or until discharge.	• 28-day mortality was 26.8% and 25% in the HCQ and standard of care groups. • HCQ treatment was markedly accompanied with an elevated length of hospital stay and elevated hazard of developing to death.
Solidarity trial[54]	An ongoing randomized controlled trial of more than 5,000 COVID-19 patients to date	HCQ Standard of care	• Not Available; Details were not published.

(*Continued*)

Table 2 (Continued).

Reference and Country	Population (n Patients)	Intervention and Comparison Groups	Primary Outcomes
US and Canada[62]	An internet-based randomized controlled trial in non-hospitalized patients in the US and Canada	HCQ(800 mg once, followed by 600 mg in 6 to 8 hours, then 600 mg daily for 4 more days) Placebo	• Symptom severity did not significantly differ over 14 days (−0.27 points (95% CI, −0.61 to 0.07 points); P=0.117). • At 14 days, 24% of the participants receiving HCQ had ongoing symptoms compared with 30% receiving placebo (P=0.21). • Medication adverse effects occurred in 43% of HCQ group compared to 22% in the placebo group (P < 0.001).

Abbreviations: HCQ, hydroxychloroquine; CQ, chloroquine; OD, one a day; BD, twice a day; TD, thrice a day; CI, confidence interval; EC50, Half maximal effective concentration; CC50, 50% cytotoxic concentration. SI, selectivity index.

systemic and discoid lupus erythematosus, sarcoidosis, scleroderma, pemphigus porphyria cutanea tarda.[63] Despite drugs' adverse effects on humans, such as cardiac, retinal, and neuromuscular toxicities, their benefits outweigh the toxicity effects.[64,65] The CQ and HCQ have also been tested to treat various diseases such as human immunodeficiency diseases, Q fever, whipple disease, and fungal infection.[65,66] These drugs have several other beneficial properties, including anti-inflammatory, immuno-modulating, anti-infective, anti-thrombotic, and anti-tumoral properties.[65]

Due to these multifaceted effects of CQ and HCQ, including antiviral properties, these drugs have been extensively investigated against the SARS-COV-2 virus and COVID-19 patients, and the outcomes widely varied. Indeed, few in vitro investigations have revealed antiviral influences against SARS-COV-2.[3,27,28] The results are preliminary based on the small clinical trials and usually cofounding with pre-existing comorbidities, age, and severity of disease.[51]

The prophylaxis use of CQ and HCQ did not show any clinical efficacy in randomized controlled trials. In most cases, there is a lack of randomized control trials with long-term supervision of the patients and their contacts to explore the efficacy of CQ/HCQ for postexposure prophylaxis. Many times, its toxicity, particularly cardiac toxicities, outweighed its benefits, unlike the treatment of malarial infection. A recent meta-analysis of 12 studies showed no evidence of clinical benefit from CQ/HCQ administration in COVID-19 patients.[35] Other limitations of this regimen were (1) the potential interaction with azithromycin and several other medications leading to QT prolongation and

possible cardiovascular side effects and (2) the hypoglycemia if not adequately monitored in diabetic patients. While close monitoring might optimize is regimen's safety, the safety profile does not make it suitable for a pandemic situation. With several cases overwhelming the healthcare systems, it becomes unpractical to screen all patients for the potential interactions in the clinical setting. Future directions in the CQ/HCQ drugs might include improved drug delivery either by inhalation[67] or transcatheter delivery through the bronchial artery.[68]

The randomized and controlled WHO Solidarity[69] trial did not find an effectiveness of HCQ in reducing mortality rate (risk ratio of 1.19; $P = 0.23$) among the hospitalized COVID-19 patients. Based on lack of benefits of using HCQ, WHO[54] and National Institute of Health had stopped trial for hospitalized COVID patients.[61] A recent randomized controlled trial by Horby et al[61] in the UK comprising 4716 COVID-19 patients showed that administration of HCQ had no benefits in decreasing death rate (rate ratio of 1.09; $P = 0.15$).

Moreover, a recent meta-analysis based on 28 randomized trial containing 10.012 COVID-19 patients treated with HCQ, 307 patients with CQ and 63 patients with both CQ and HCQ in which WHO Solidarity[69] and RECOVERY[61] included that HCQ treatment was associated with increased (risk ratio of 1.11; $P = 0.02$) mortality rate, whereas CQ did not show (risk ratio of 1.77; $P = 0.21$) any benefit in reducing mortality rate.[70] Finally, according to new data from two large RCTs (Recovery and Solidarity), the United States Food and Drug Administration (FDA) revoked the CQ/HCQ regimen's emergency usage authorization in COVID-19 patients.

The drugs are currently used for clinical trial purposes only.[21] Furthermore, we searched clinicaltrials.gov for clinical trials on COVID-19 using the keywords: "chloroquine OR hydroxychloroquine". Then, we filtered the records to identify the "ongoing studies only". The search retrieved 97 ongoing studies; the summary of the 97 ongoing studies and their characteristics are provided in the Supplementary Table S1.

Conclusion and Recommendations

Based on the initial early experimental data of CQ and HCQ for treatment of SARS-CoV-2, the regimen received an emergency usage authorization from the FDA for COVID-19 on March 28, 2020. However, the two largest RCTs data to date showed no clinical advantage of HCQ treatment in COVID-19 patients. As a result, the FDA revoked the emergency use authorization of this regimen. In terms of prophylaxis, one RCT showed no evidence of post-exposure prevention from COVID-19. Despite the initial promising findings in the in vitro studies and the widespread use of CQ/HCQ in clinical settings during the 1st wave of COVID-19, current data from well-designed randomized controlled trials showed no evidence of benefit from CQ/HCQ supplementation for the treatment or prophylaxis against SARS-CoV-2 infection. Particularly, the two largest randomized controlled trials to date (RECOVERY[61] and WHO SOLIDARITY[69]), both confirmed that CQ/HCQ regimen does not provide any clinical benefit for COVID-19 patients. Therefore, we do not recommend the use of this regimen in COVID-19 patients outside the context of clinical trials.

Data Sharing Statement

This review article is based on the published available literature.

Author Contributions

All authors (S.S., N.A., M.A., Y.A., S.A., S.S.E., M.S., O. A., N.A., A.A., O.A., A.K.P., A.K., A.N., A.N., W.A-A., A.A.A., K.A.E.-T. and M. A.E.-H.) have made a significant contribution to this review article. They all equally shared in the conception, study design, execution, acquisition of data, analysis and interpretation. S.S., N.A., M.A., Y.A., S.A., S.S.E., K.A.E.-T, and M.A.E.-H. have drafted the manuscript. M.S., O.A., N.A., A.A., O.A., A.K. P., A.K., A.N., A.N., W.A-A., A.A.A., K.A.E.-T. have substantially revised and critically reviewed the article. All authors have agreed on the submission to this journal and agreed on all versions of the article before submission

or during revisions. All authors agreed to take responsibility and be accountable for the contents of this article.

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