Exploring insights of hydroxychloroquine, a controversial drug in Covid-19: An update

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ARTICLE INFO

ABSTRACT

The review summarizes chloroquine (CQ) and its safer derivative hydroxychloroquine (HCQ) and its utility in Covid-19. Recently this well-established drug made its way back to the headlines during the SARS-CoV-2 pandemic. This led to an upsurge in the scientific arena with multiple research and review articles along with expert opinions and commentaries. The HCQ has received mixed judgements so far about its efficacy to be used in Covid-19 patients in a limited trial conducted all across the Globe. The purpose of our article is to put forth the history, pharmacodynamics, and pharmacokinetics, along with the existing studies favouring and disapproving the role of HCQ in the treatment of Covid-19. We grouped HCQ use at three stages, this includes HCQ for **i.** prophylactic use by asymptomatic health workers or peoples at higher risk; **ii.** patients having mild symptoms; **iii.** patients with extreme symptoms. The review critically discusses the underlying plausible reasons and mechanisms exploring HCQ in prophylactic management or treatment of SARS-CoV-2. Furthermore, we have critically analysed the reported pharmacokinetic parameters and compiled the proponent, opponent, or neutral opinions on the use of HCQ in Covid-19. Authors discretion is to conduct more studies considering the optimal dosing regimen and pharmacokinetics assessment.

1. Introduction

Coronavirus disease 2019 (Covid-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a pandemic disease that has deteriorated the World in terms of health and wealth. Considering the treatment, the World is still deprived of an efficacious drug or a vaccine to combat Covid-19 (Poduri et al., 2020). Since SARS-CoV-2 has left the World unparalleled and unbiased in this pandemic, this has driven the scientific community to go all out to search for a solution (Thakur et al., 2021). Considering the scientific advancements, many drugs are being repurposed for testing against SARS-CoV-2 but with little success so far as the World awaits its first USFDA drug approval for Covid-19 (Qian et al., 2020). Among hundreds of drugs repurposed, chloroquine (CQ) and its safer derivative hydroxychloroquine (HCQ) have been explored for their role in SARS-CoV-2 owing to their prior use in SARS-CoV and also considering the much higher similarity in two virus strains (Frie and Gbinigie, 2020; Spinelli et al., 2020). CQ and HCQ are medications that have been used for a long time. The USFDA first

approved HCQ on 18 April 1955 for the treatment of malaria. HCQ has been further explored as an immunomodulator in treating autoimmune diseases, including lupus erythematosus and rheumatoid arthritis. It is also known to possess antiviral activity for hepatitis B, HIV, H1N1 and Zika virus (Browning, 2014; D'Alessandro et al., 2020). The drug is reported to act in 392 diseases. Recently this well-established drug made its way back to the headlines during the SARS-CoV-2 pandemic and was further fuelled by the United States of America's President Donald Trump, calling it a "game-changer"(Downes et al., 2020). This has led to an upsurge in the scientific arena with multiple research and review articles and expert opinions and commentaries. 'Scopus' search with the word "hydroxychloroquine" yielded 26853 results. The first results appeared in the year 1946, with 6443 articles published to date in the year 2021 (February 16, 2021). The advanced search, i.e., "Hydroxychloroquine" AND "Covid-19," yielded 4396 publications (as of February 16, 2021), suggesting the drug has vastly been explored for the current pandemic (see Fig. 1). The purpose of this article is to put forth the history, pharmacodynamics, and pharmacokinetics, along with the

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existing studies favouring and disapproving the role of HCQ in the treatment of Covid-19. The paper discusses the underlying plausible reasons and mechanisms exploring HCQ in prophylactic management or treatment of SARS-CoV-2. Furthermore, we have critically analysed the reported pharmacokinetic parameters and compiled the proponent, opponent, or neutral opinions on the use of HCQ in Covid-19.

2. The underlying philosophy: Timeline

The focus on CQ/HCQ therapy for SARS-CoV-2 first came to attention in early February after many journals published reports on *in vitro* efficacy of CQ. Further, the State Council of China, on 17 February 2020, disclosed the efficacy of CQ in the treatment of pneumonia associated with Covid-19 (Chen et al., 2020a). The results were the outcome of a multicentre, non-randomized clinical trial conducted in China. This further led to some more clinical trials in China and other parts of the Globe. In total, nine clinical trials were conducted to date for CQ after the pandemic, out of which a clinical trial of significant note for CQ includes a trial by Gao and group. In this study, the treatment of CQ improved exacerbation of pneumonia, improved lung imaging, and shortened the duration of disease in 100 patients with worsening cases of pneumonia. However, the study did not disclose the data for the claims made (Gao et al., 2020).

Later, CQ was replaced with safer analogue HCQ based on its use in previous pandemics, particularly SARS-CoV. To date, it is repositioning for SARS-CoV-2 was justified by preliminary studies made by Yao and his group. The group disclosed a comparative *in vitro* study using CQ and HCQ and found HCQ superior in treating Covid-19 (Yao et al., 2020). The group recommended 400 mg for day one, followed by 200 mg for the next four consecutive days. The findings were later validated by a study published by the Wuhan Institute of Virology, which was further corroborated by the study of Devaux and group who emphasized the use of HCQ for curative prophylaxis treatment (Devaux et al., 2020). First, *in vivo* evidence for the efficacy of HCQ was disclosed by Gautret et al. in a non-randomized clinical trial. The research disclosed that the use of HCQ (200 mg) 3 times a day for ten days (with or without azithromycin) starts to eliminate the virus from the sixth day (Gautret et al., 2020a,b). This very study was claimed to be a "game-changer" by the USA president Donald Trump and, at the same time, was endorsed by many institutional/Government leaders. As a consequence of this, the data accessed on the Clinicaltrial.gov portal, about 271 clinical trials are undergoing or completed to date (February 16, 2021) world-wide for exploring the potential of HCQ for SARS-CoV-2.

To date, enough rationale exists to justify the efficacy and safety of HCQ in Covid-19 (Hashem et al., 2020). However, evidence of the effects is limited. If the trials under progress establish the efficacy of HCQ for either prophylaxis or treatment of Covid-19, it would be advisable to define, record, and maintain the drug doses, keeping into consideration age, sex, obesity, and other comorbid conditions thus concerning the importance of triage.

3. Delving deeper into the pharmacodynamics of CQ/HCQ in Covid-19: Molecular mechanisms involved

Chloroquine (CQ) and Hydroxychloroquine (HCQ) chemically belongs to the 4-aminoquinolones class (Browning, 2014). Chloroquine, first discovered in 1934 by the pharmaceutical company Bayer was approved in 1949 to treat malaria and amebiasis (Coatney, 1963; Krafts et al., 2012). Nevertheless, due to arousal in cases of overdose of CQ, leading to acute poisoning and death, its market value had decreased and was soon replaced with HCQ (Frisk-Holmberg et al., 1979; Weniger, 1979). HCQ is much more efficacious and showed ~40% less toxicity in animal models compared to CQ. HCQ was first synthesized in 1946 with the incorporation of the hydroxyl group into CQ during World War II (Hoekenga, 1955; Surrey, 1951). The first report on its synthesis was published in 1950 by two chemists Alexander Surrey and Henry Hammer, working at the Sterling–Winthrop Research Institute (Rensselaer, NY). The company obtained a US patent in the same year for the compound and its synthesis (Surrey, 1951). HCQ is chemically 2-[[4-[(7-Chloro-4-quinolyl)amino]pentyl] ethylamino]ethanol with sulfate salt (2020). It possesses a flat aromatic core structure and exists as a racemic mixture with an R and S configuration. The (R)-$(-)$-hydroxychloroquine form is reported to be present at higher concentrations in the blood than the (S)-$(+)$-hydroxychloroquine form. Both forms have been found to play differential deposition and/or metabolism with similar pharmacodynamic effects. HCQ is a weak base due to the availability of a basic side chain supported by the hydroxy group. The side chain is responsible for the accretion of the drug in lysosomal compartments and is of utmost importance for its efficacy and interaction with nucleic acids (Soria, 2016).

Although the precise mechanism of action of CQ/HCQ is not established, these drugs produce effects at molecular and cellular levels (Noël and Lima, 2020). The former involves inhibition of lysosomal activity, autophagy and signalling pathways, while the latter involves inhibition of cytokine production and immune activation. During the interaction of SARS-CoV-2 with the host cell, the first step involves cleavage of S protein of the virus with a transmembrane serine protease, TMPRSS2, which activates S protein allowing the virus attachment with ACE2,
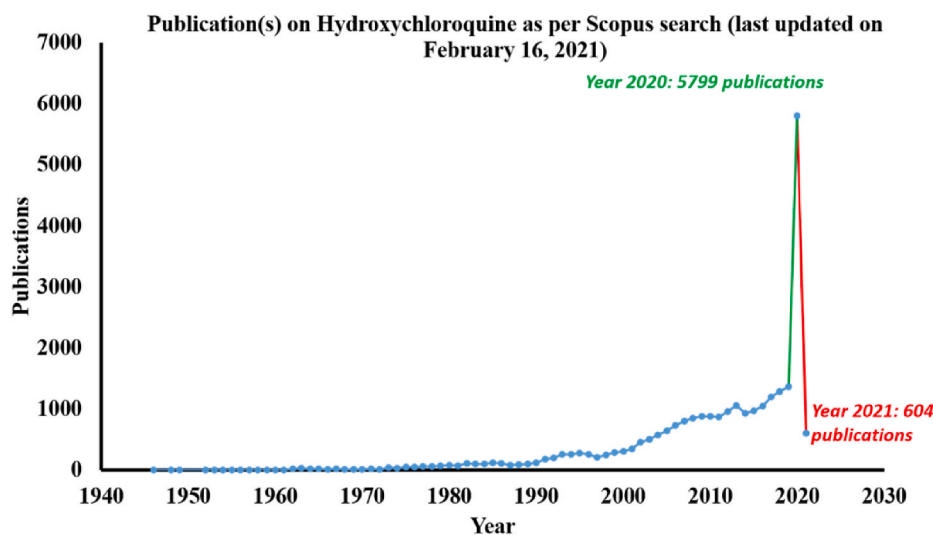

Fig. 1. Graph suggesting the total number of publications for key search "Hydroxychloroquine" using Scopus (Accessed on February 16, 2021).

which acts as an entry receptor for SARS-CoV-2 (Ou et al., 2020). CQ/HCQ are reported to exhibit their antiviral activity via multiple pathways. The critical pathways include i) inhibit viral entry; ii) uncoating; iii) interference with terminal glycosylation of ACE2; iv) proteolytic maturation of proteins and v) increase in the pH of endosomes, Golgi vesicles, and lysosomes; vi) assembly and budding (Browning, 2014; Homewood et al., 1972; Thomé et al., 2013). These events inhibit the viral release into the host cell playing the preventive role. Besides, CQ/HCQ is purported to combat Covid-19 by its immunomodulatory properties. The drug is reported to i) inhibit cytokine production and its release by T-cells, which lead to cytokine storm; ii) inhibit lymphocyte activities of $CD4^+$ and cytotoxic T cell; iii) decrease in the levels of chemokines CCL2 and CXCL10; iv) inhibit Treg and IFN-α activities (Schrezenmeier and Dorner, 2020). Mechanistically, CQ/HCQ accumulates in lysosomes and destabilizes the pH gradient leading to inhibition of lysosomal protease that requires acidic pH for optimum functioning. As both CQ/HCQ are weak bases, they elevate the pH of endosomes/lysosomes from 4.5 to 6.5 at 100 μM. Another mechanism reported that inhibiting viral entry is via inhibition of quinone reductase. This enzyme plays a significant role in the biosynthesis of sialic acid. Sialic acid is known to assist in virus-host cell recognition. Based on *in-silico* investigations, Fantini and the group reported that SARS-CoV-2, in addition to the ACE2 receptor for entry, may use host cell sialic acids linked to gangliosides at the host cell surface and improve cellular attachment (Fantini et al., 2020). CQ/HCQ reduces phosphatidylinositol binding clathrin assembly protein (PICALM) expression that plays a vital role as a cargo-selecting adaptor and regulates the rate of cellular clathrin-mediated endocytosis assisting SARS-CoV-2 entry (Inoue et al., 2007; Wolfram et al., 2017). Besides, CQ/HCQ is reported to possess zinc ionophore physiognomies and thereby specifically target extracellular trace element zinc and allow its intracellular transit to lysosomes where it interferes explicitly with Nsp-12 (RNA-dependent RNA polymerase; RdRp) activity and consequently blocks SARS-CoV-2 replication. Zinc is already reported to enhance antiviral immunity (Shittu and Afolami, 2020; Xue et al., 2014).

Some studies underlie the effect of CQ/HCQ that may allow cellular iron starvation in the virus and thereby inhibit SARS-CoV-2 replication. Mechanistically, CQ/HCQ inhibits the iron-regulatory hormone hepcidin (HAMP), known to block cellular iron export mediated via ferroportin1 (FPN1). This results in reduced iron absorption, increasing iron retention in hepatocytes and macrophages; thus, provoking infection/inflammation. HAMP is produced in the immune system (lymphocytes, monocytes, and macrophages along with alveolar macrophages) and airway epithelial cells and have been reported to contribute to lung injury (Roldan et al., 2020). Furthermore, considering lysosomes, which own hydrolytic enzymes that mediate autophagy or endocytosis pathways. Thus, CQ/HCQ interference with lysosomal activity is thought to inhibit/alter the functions of lymphocytes leading to anti-inflammatory or immunomodulatory effects (Adeel, 2020). Importantly, lysosomes are also indirectly involved in immune system activation by antigen processing via $CD4^+$ T-cells and histocompatibility factors activation (MHC II), leading to autophagy (Alijotas-Reig et al., 2020). Thus CQ/HCQ is also known to inhibit lysosomal and autophagosome functions that activate the immune system indirectly (Mauthe et al., 2018). The studies also report that these drugs also lead to the downregulation of TLR receptors. Upon accumulation of drugs in host cell endosomes, the alteration in pH hampers the TLR processing. Also, these drugs bind to double-stranded DNAs minor groove and prevent ligand binding to TLR7 (RNA) and TLR9 (DNA) (Müller-Calleja et al., 2017; Torigoe et al., 2018). The HCQ is also reported to inhibit Cyclic guanosine monophosphate–adenosine (cGAMP) synthase activity, which acts as a stimulator of interferon (type I IFNs) genes (An et al., 2015). The drug inhibits cGAMP synthase-dependent transcription of type I IFNs by binding with cytosolic DNA via transcription factor IFN regulatory factor 3 (IRF3). Thus, via combinatorial inhibition of TLR and cGAMP synthase, it reduces the production of proinflammatory cytokines along

with including type I interferons. Some *in vitro* reports suggest that CQ/HCQ inhibits IL-1, IL-6, TNF, and IFNγ production (An et al., 2018). One recent study has also identified that drug also interferes with lipid-modified proteins' catabolism by inhibiting palmitoyl-protein thioesterase 1 (PPT1) overexpressed in the synovial tissue of patients with Rheumatoid arthritis (Rebecca et al., 2019; Schrezenmeier and Dörner, 2020). Further attempt to delineate the plausible mechanism of HCQ was attempted using computational approaches. The basic sketch to illustrate the mechanism of CQ/HCQ in Covid-19 is provided in Fig. 2. Beyond Covid-19, CQ and HCQ have also been explored to treat various other diseases and infections (Gies et al., 2020). The important utility of these drugs has been explored in human malaria, hepatic amebiasis, lupus erythematosus, rheumatoid arthritis, Porphyria cutanea tarda, primary Sjögren syndrome, Q fever, Sarcoidosis and dermatomyositis. Besides antiviral effects, these drugs have also been known to possess antibacterial, antifungal, antiprotozoal and antiparasitic assets. Further few *in vitro* and *in vivo* studies have highlighted their use in several forms of cancer, glioblastoma along with possessing immunomodulatory effects. Considering their pivotal immunomodulatory and anti-inflammatory potentials, CQ and HCQ are known to inhibit TLRs (TLR-3,7, 8 and 9); Interferons (IFN-α, IFN-γ); T-cells (Th1, 2 and 17); TNF-α and interleukins (IL-1, 2, 6, 17, 22) and act as a facilitator for IL-10. Among numerous ILs effected by CQ/HCQ IL-1, IL-1β cell concentration was found to get reduced by CQ in rheumatoid arthritis patients by interfering with endolysosome-associated vesicles mediated pathway in monocytes (Gasmi et al., 2021; Gies et al., 2020). CQ is also known to inhibit IL-2 by modulating αCD3 in MoAb-triggered T-cells plausibly in an autocrine fashion. However, inhibition of IL-2 does not affect the secretory concentration of IL-2 receptor complex. Further, the synthesis of IL-6, a pleiotropic cytokine and plays a role in B-cell maturation, is known to inhibit CQ. HCQ is known to inhibit IL-17 and IL-22 and consequently reduces Th-17 cytokine levels and antigen presentation. Further few other inhibitors have also been explored for their utility as anti-inflammatory agents, thus proving their utility in Covid-19 (dos Reis Neto et al., 2020b). A recent study by Ignaitos and group disclosed the beneficial effect of Tocilizumab on endothelial glycocalyx and myocardial efficiency via IL-6 inhibition in rheumatoid arthritis patients. The favourable outcomes like improvement of vascular permeability in these patients were correlated with an apparent beneficial effect in Covid-19, characterized by excess IL-6 release (Ikonomidis et al., 2020). Lambadiari and the group explored the possibilities and mechanisms for higher risk of Covid-19 in diabetic patients. The team hypothesised that diabetes-associated hyperactivation of NLRP3 (NOD-, LRR- and pyrin domain-containing protein 3) inflammasome, chronic inflammation, hypercytokinemia, followed by increased vascular permeability might majorly contribute to the severity of Covid-19. The group also advised using colchicine, anti-IL1a, anti-IL1β, or anti-IL6 as a therapeutic intervention in overcoming the associated complications in Covid-19 (Lambadiari et al., 2020). In another research by Korakas et al. explored the correlation between obesity and Covid-19. The group disclosed the excess deposition of adipose tissue in obesity could provoke an acute hyperinflammatory state similar to that characterized in Covid-19. Thus, diseases with similar inflammatory profiles could be mechanistically overviewed for better and safe therapeutic interventions against Covid-19 (Korakas et al., 2020).

At present, it is debatable and unclear whether CQ/HCQ has any role for prophylaxis or treatment of Covid-19, or it is merely a placebo. The reason for this impasse is based on a large number of *in vitro* studies, which are typically favourable relative to limited clinical studies that are not reproducible (Levantovsky and Vabret, 2020). Additionally, some toxicities are reported for the drug, which notably include effects on the heart leading to QRS and QT interval prolongation via sodium and potassium channel blockade, causing dysrhythmias and ventricular fibrillation. Besides some ophthalmologic complications (retinopathy), hypoglycemia and death at overdoses are reported (Downes et al., 2020). The major complication lies with improper monitoring of doses
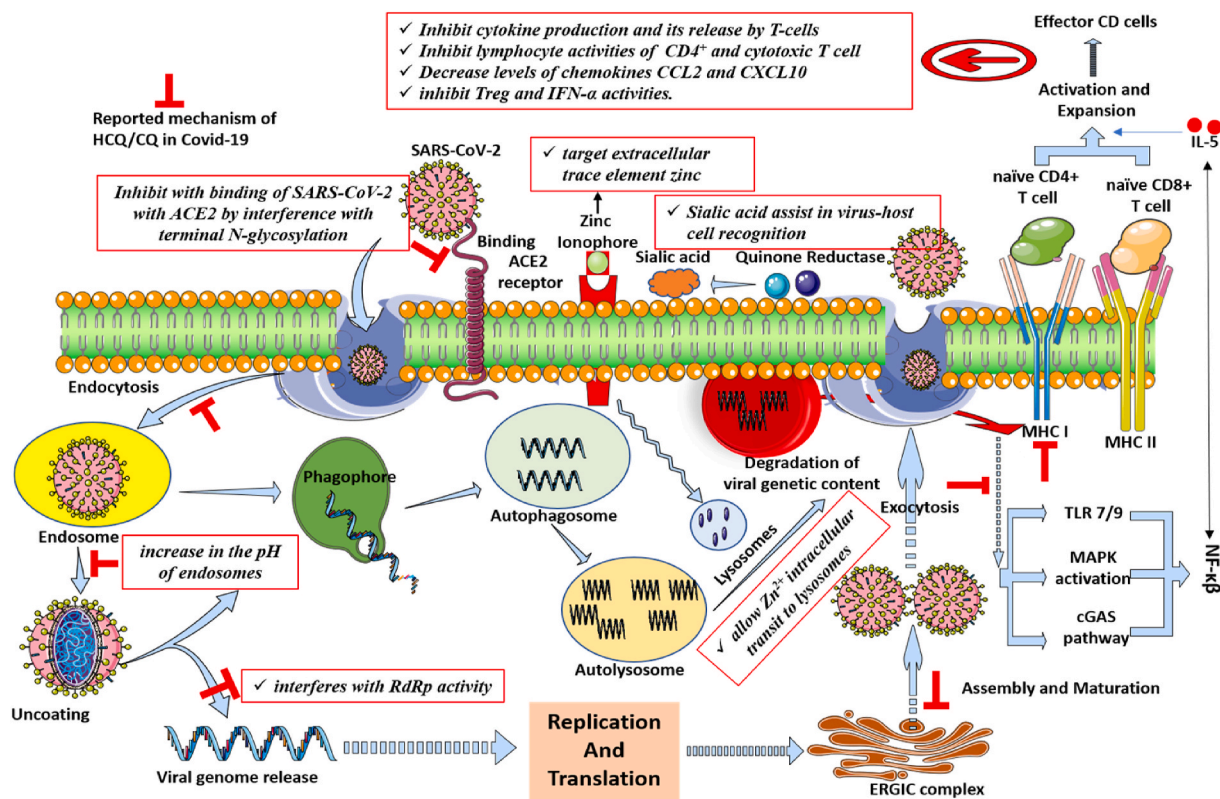
Fig. 2. Pictorial representation of the mechanism of action of CQ/HCQ in Covid-19.

as it severely depends on its pharmacokinetics (Liu et al., 2020). This could be minimized by appropriate drug dosage, considering essential parameters such as body weight and disease condition.

4. The implication of pharmacokinetics of HCQ and its role in manipulating its pharmacodynamics

Understanding the dose-response relationships due to their complex pharmacokinetics and an extensive volume of distribution. Further different pharmacokinetic profiling may vary in separate compartments that include plasma and include blood and serum of the individual patient. CQ and HCQ are administered as phosphate and sulfate salt, respectively. Drug absorption takes place in the upper intestinal tract. CQ/HCQ is readily absorbed (mean Tmax 0.43 h) and possesses an excellent oral bioavailability (70–80% following 200 mg dose). The uneven distribution of the drug in the body is influenced by the dosage that varies from patients to patients as it is dependent on the patient's disease condition, which in turn affects its half-life and elimination kinetics (Collins et al., 2018; dos Reis Neto et al., 2020a). The drug is known to distribute well with prolonged retention in melanin, liver, skin, heart, lungs, and choroid and ciliary bodies of the eye. The pre-clinical experiments (measured throughout 168 h followed by a single dose) have suggested the distribution concentration is highest in lungs (11.8–450 times), heart (6.8–184 times), erythrocytes (7.3–10.4 times), and blood (2.5–8 times) compared to drug concentration in plasma. The significant fluctuation in the volume of distribution (V_d) is reported by several researchers based on their experimental setups (Miller et al., 1991; Schrezenmeier and Dörner, 2020). Cutler et al. have reported a large V_d of 47,257 L for HCQ and 65,000 L for chloroquine in the blood with a half-life of 40–60 days on average (Cutler et al., 1988). In a study by Tett et al. five healthy volunteers receiving an intravenous infusion of 155 mg HCQ each. The V_d was calculated to be 5522 L in blood and 44,257 L in plasma with a mean half-life of 40 days with variable bioavailability in all the volunteers. Although the study reported a

constant therapeutic dose, it caused variable blood to plasma concentration ratios. The authors suggest measuring whole blood concentration rather than plasma concentration in reporting HCQ pharmacokinetics. A similar trend was noted for CQ (Tett et al., 1988). Lim et al. analysed the V_d of HCQ in the South Korean population treated for malaria with an oral dose of 200 mg HCQ. They reported blood and plasma V_d of HCQ 733 L and 1630 L, respectively (Lim et al., 2009). Based on these study findings, a dose range of HCQ in the treatment of Covid-19 that follows linear pharmacokinetics is to be determined. The V_d depends on the extent of protein binding, a rate-limiting step in eliminating the drug from the human body. The average percentage of unbound HCQ was 50% (Furst, 1996; Smit et al., 2020).

Cytochrome P450 (CYP) isoenzymes, CYP1A2, CYP2C8, CYP2C19, CYP2D6, CYP3A4/5, CYP2C8, and CYP3A4/5, catalyses the dealkylation of CQ/HCQ, leading to the formation of active metabolites desethylchloroquine (common metabolite for both CQ/HCQ) and desethylhydroxychloroquine, respectively. The effectiveness of desethylchloroquine in Covid-19 is unknown (Smit et al., 2020; Yazdany and Kim, 2020). These metabolites undergo further metabolism to a toxic metabolite, bisdesethylchloroquine, which is reported to cause heart failure after long-term use (Karunajeewa et al., 2010). A single dose of HCQ and CQ is cleared via the renal route to the extent of 40–50% and 57%, respectively. Published studies have shown that the plasma concentrations of HCQ useful in malaria were achieved in treating patients with Covid-19. Similar QTc measurements were considered during pre-and post-treatment in both diseases. However, HCQ was found not effective in preventing, treating, or slowing the disease's progression in most of the studies reported so far (Lim et al., 2009). The plausible reason for the failure of HCQ in Covid-19 despite showing promising result at onset may be attributed to some of its pharmacokinetics that is poorly understood, These include, i) combination treatment strategies with HCQ that can potentially prolong QTc interval; ii) patients with cardiac complications; iii) body mass and fluid content; iv) co-disease conditions along with Covid-19; v) status of microsomal enzymes and

concomitant drugs administered with CQ/HCQ, particularly CYPs inhibitors; vi) Plasma protein binding, and vii) renal dysfunction (Yazdany and Kim, 2020). Moreover, no actual effort has yet been made in deciphering the exact dose of CQ/HCQ in mitigating Covid-19 either prophylactically or for its treatment. The current dosage knowledge is derived only from the studies made on these drugs on healthy volunteers or in other non-SARS-CoV-2 cases (Garcia-Cremades et al., 2020). The significant pharmacokinetics correlation made for HCQ in Covid-19 is compiled in Table 1.

The critical drawbacks from the above table, considering the critical gaps and inconsistencies in the literature are **i.** Variability in dosing of HCQ and route of administration is not defined. Besides, some studies are involved using crushed HCQ for feeding to patients via tube, though HCQ is a film-coated drug listed in ''Do Not Crush'' medication list (Mitchell, 2011); **ii.** uncertainty in absorption following various routes, with indefinite bioavailability; **iii.** Distribution studies are confined to animal models, which will highly differ in humans due to lower drug recovery rates and metabolic patterns of drugs as influenced by Cyps; **iv.** no research investigating genetic association (notably with CYP3A, 2D6; and 2C8) monitoring HCQ drug levels in patients have been done so far; **v.** No data on drug transporters associated with HCQ or excretion data have been reported so far for Covid-19.

Table 1
Current status of pharmacokinetics studies conducted on HCQ.

Study	Findings	Ref
Dosing Regimen		
A multicenter, retrospective, observational analysis of hospitalized Covid-19 patients	HCQ 400 mg twice daily on day 1, followed by HCQ 200 mg twice daily on days 2–5	Arshad et al. (2020); Geleris et al. (2020)
Solidarity trial on HCQ arm by WHO	HCQ 800 mg twice daily on day 1, followed by HCQ 400 mg twice daily for 10 days	Organization (2020)
Absorption		
In healthy males who received a single HCQ 200 mg oral dose,	➢ Peak concentrations observed within 3–5 h ➢ Mean peak blood HCQ concentration was 0.1296 mcg/ml in 3.26 h ➢ Peak plasma HCQ concentration was 0.0503 mcg/ml achieved in 3.74 h.	Morrisette et al. (2020)
Randomized, crossover study with HCQ 155 mg oral tablet was compared with intravenous infusion of racemic HCQ 155 mg	From oral dose, absorption was 0.74 ($\pm$0.13), while high variability was seen in plasma data. The data was estimated using and blood	Morrisette et al. (2020); Tett et al. (1989)
Tissue Distribution		
No human studies have been made so far in Covid-19. One animal study on macaques was conducted for HCQ	Tissue distribution suggested drug concentration chiefly in lungs and kidney	Maisonnasse et al. (2020)
Metabolism		
Desethylhydroxychloroquine (major), desethylchloroquine, and bisdesethylhydroxychloroquine are three metabolites generated from HCQ.	It is still unclear how these metabolites confer activity against SARS-CoV-2. Presence of significant metabolite in Covid-19 patients upon administration of CQ/HCQ is unclear	Bauman and Tisdale (2020); Morrisette et al. (2020)

Further, recently the pharmacokinetics-based studies were performed for HCQ in Covid-19 patient by Perinel and group. The work suggested a mix of scientific experiments followed by model-based analysis to predict optimized efficacy and safe dose for HCQ. In the study, 13 patients were included comprising 12 mechanically ventilated patients (median weight: 82.7 kg, median age: 68 years). The initial dose was given was 200 mg t.i.d via the oral route that led only 61% of patients in the study to achieve therapeutic levels (1 mcg/ml), and 15% were found to acquire toxic levels (2 mcg/ml). Further, based on these initial inputs, a simulation study was done to decide an optimal dosing regimen. The analysis revealed with 800 mg of loading dose on the first day followed with 200 mg bid for 7 days, provide optimal effects (Perinel et al., 2020).

5. The plethora of evidence 'for' and 'against' the use of HCQ in Covid-19: A study of cases

On March 28th, 2020, the US FDA issued an Emergency Use Authorization for the use of HCQ (and CQ). The FDA quoted, "*HCQ sulfate may only be used to treat adult, and adolescent patients who weigh* 50 kg *or more and are hospitalized with Covid-19, for whom a clinical trial is not available, or participation is not feasible*" . However, the US FDA revoked the authorized use of HCQ/CQ recently on June 15, 2020. However, there is much debate on whether to use these drugs for Covid-19. So, what has happened during these two and a half months that is before the status of CQ/HCQ was revoked? Before going further, let us consider the essential and vital areas in the use of HCQ in Covid-19. The important ones are, (i) patients were treated at high doses of 600–800 mg for a short duration of 10 days. This will not lead to high risk unless the patients show ADRs as a result of co-morbidities/susceptibility to HCQ, including retinopathy or diabetes; (ii) HCQ in trials for prophylaxis that could be presumably for a longer duration with unknown doses. This would be opening the risk for retinal toxicity depending on the dose, (iii) self-medication without a knowledge of the dose, duration, and adverse effects. This opens up high risk, probably due to no monitoring of doses and toxicities as a consequence; (iv) postponement of HCQ monitoring by reason of improper coordination or lockdown or some unknown reason with a postponement of appointments. This is expected and could be of low risk if proper follow-ups are taken for any unwanted effects they are experiencing, including deterioration of vision (Colson et al., 2020; Ferner and Aronson, 2020; Yazdany and Kim, 2020; Zhou et al., 2020).

USFDA authorized the use of CQ/HCQ based on a large amount of data from *in vitro* studies and with limited clinical evidence. A first report published by Wang et al. reported the efficacy of CQ in potentially blocking SARS-CoV-2 infection using Vero cells (treated with CQ for 48 h) with an EC50 of 1.13 μM (clinically achievable at a dose of 500 mg/day). The finding was corroborated using RT-PCR, immunofluorescence microscopy followed with immunoblotting assays (Wang et al., 2020). Further, the group led by Liu et al. compared CQ/HCQ using similar studies and concluded that CQ is more efficacious than HCQ (5.47 and 6.14 μM, respectively) with a similar mechanism of blocking endocytotic vesicle maturation, that is, blocking infection with SARS-CoV-2 at both entry and post-entry levels (Liu et al., 2020). However, Yao et al. reported HCQ was statistically significantly more potent than CQ (0.72 and 5.47 μM, respectively) in in vitro studies) (Yao et al., 2020). There have been limited *in vivo* studies elucidating the mechanism of HCQ in SARS-CoV-2 infection. Clinical study results on HCQ in Covid-19 patients were first briefed in February 2020 by the Chinese government. It was revealed that a significant improvement of pneumonia and lung imaging, along with a reduction in the span of illness, was observed in 100 patients treated with CQ with no observable adverse effects. The dose suggested in the study was 300 mg for CQ twice a day, for 10 days (Gao et al., 2020). This was further followed by another Chinese study in which HCQ (in comparison to placebo) could reduce the time to clinical recovery in 62 patients (Chen et al., 2020b). The first report on

non-randomized clinical trial came was published on March 17, 2020, by Gautret et al and group. The study was an open-label non-randomized controlled trial in 36 patients. The treated group was categorized further based upon the symptomatic conditions of upper respiratory (22 patients) or lower respiratory tract infections (8 patients) and asymptomatic (6 patients). A total of 20 patients received HCQ 200 mg three times a day for ten days, and 6 of these were given HCQ with azithromycin (to avoid bacterial superinfection) and rest control group was given usual care. The outcome of treatment was analysed on the 6th day, suggested a large number of patients in the treatment group were found negative (70%) compared to the control group (12.5%). Patients receiving combination dose were all tested negative (Gautret et al., 2020a). Besides, on the same day, i.e., March 17, 2020, National Institute for the Infectious Diseases "L. Spallanzani" IRCCS in Italy published their recommendations for use of HCQ (400 mg/day) and CQ (500 mg/day) along with another antiviral drug in combination for the treatment Covid-19 (Nicastri et al., 2020).

These encouraging results further supported the Indian Council of Medical Research (ICMR) to recommend the use of HCQ for chemo-prophylaxis on March 22, 2020. The guidelines stated the use of HCQ for asymptomatic health workers (400 mg twice on day 1, followed by 400 mg once every week for 7 weeks) or household contacts of positive patients (400 mg twice on day 1, followed by 400 mg once every week for 3 weeks) with SARS-CoV-2. Taking the lead from all the positive findings, the USFDA authorized emergency use for HCQ on March 28, 2020, to treat COVID-19 associated pneumonia (Lenzer, 2020) followed by the Indian Council for Medical Research who recommended for empiric use of HCQ for Prophylaxis of SARS-CoV-2 Infection (Chauhan et al., 2020). There are reports that point toward the failure in using HCQ therapy for treating Covid-19. In this context, Ferner and Aronson reported the use of CQ/HCQ in Covid-19 is "*premature and potentially harmful.*" However, they agreed that disparity between laboratory and clinical experiments is mainly due to complex pharmacokinetics and hence makes it difficult to extrapolate drug concentrations in culture media to human doses (Ferner and Aronson, 2020). Furthermore, Molina and co-researchers reported no evidence of rapid antiviral clearance or clinical benefit with the combination of HCQ and azithromycin in patients with severe COVID-19 infection. Their conclusion was based on a prospective study on 11 patients (7 men and 4 women, having complications of obesity (8), solid cancer (2), haematological cancer (3): HIV-infection (2) (Molina et al., 2020). Later, using the same dosing regimen, Gautret et al. noted the virologic and clinical outcomes. They found, within the treatment of 5 days, one patient died, and rest were in possession of fever and too received nasal oxygen therapy, among which two were further referred to ICU, and one was found to be with prolonged QT interval (Gautret et al., 2020a). A multinational Network Cohort and Self-Controlled case series study were conducted by Lane and group on the use of HCQ alone or in combination with azithromycin. The study observed an increase in QT interval prolongation leading to cardiovascular adverse event and death. Despite this adverse event, the group favoured short term HCQ use without the use of azithromycin in combination since ADR severity was found to higher under the synergistic use of both drugs. The study also highlighted the need for different dosage regimens and duration of treatment (Lane et al., 2020). The prospects for the use of CQ/HCQ were further dampened by the failure of the RECOVERY Trial, which suggested the drug is not effective in Covid-19 hospitalized patients. The study included recruitment of 1542 patients treated with HCQ, and 3132 received usual care, and it was found there was no significant difference in mortality analysed on the 28th day (25.7% deaths in patients receiving HCQ and 23.5% with usual care) (Horby and Landray, 2020). Besides, there are many other upcoming studies which are pointing toward similar outcomes (Mahevas et al., 2020; Molina et al., 2020). Based on these results, the USFDA revoked the status of HCQ in treatment for Covid-19 from its global drug trials. However, the ban was imposed based on trial results from in-hospital patients; however, out-patients results are still expected

probably by September 2020. An outpatient study recently conducted by Caleb and group also pointed that HCQ do not found much active in reducing the severity of symptoms severity in Covid-19 patients. The assigned dose was 800 mg once, followed by 600 mg in 6–8 h on same day, further followed by 600 mg 4 days in comparison to placebo on 491 patients (Skipper et al., 2020). Recently some ray of hope was still published after the WHO revoked the use of HCQ alone or in combination. A study by Arshad et al. discussed the positive role of HCQ alone or in combination with azithromycin or azithromycin alone in comparison to placebo during their multicentre retrospective observational study in hospitalized Covid-19 patients. HCQ was dosed at 400 mg (b.i.d) for day 1, followed by 200 mg (b.i.d) for next 2–5 days. The Azithromycin was dosed at 500 mg for day 1 followed by 250 mg for the next 4 days. The combination was reserved for patients with minimal cardiac risk factors. The study was made on 2541 patients (2948 recruited) with a median age range of 53–76 years, with 51% male and 49% female. Out of total patients, the 18.1% mortality rates were observed in the entire cohort, 13.5% in the HCQ group (162 patients out of 1202), 20.1% in the combination treatment group (157 patients out of 783), 22.4% in the azithromycin group (33 patients out of 147) and 26.4% in patients receiving none of the prescribed regime (108 patients out of 409). The primary cause of death in 88% was found to be a respiratory failure, whereas no death was found to occur via abnormal heart rhythm (Arshad et al., 2020). Further, a new ray of hope is provided on June 30, 2020, by the Medicines and Healthcare products Regulatory (MHRA) Agency that is restarting its pending trial (COPCOV) on HCQ to explore its prophylactic potential. The pending study involved a randomized, placebo-controlled trial and will be acquiring 40,000 healthcare workers and risk staff throughout the Globe. In a similar line, the Minnesota study during triad of independent randomized controlled trials suggested no efficacy of HCQ on symptomatic patients, however efficacy of HCQ for prophylaxis use on health workers is yet to be published (Boulware et al., 2020). Further, in their editorial, the group disclosed three important conclusions, **i.** HCQ might be effective in early treatment, **ii.** need to conduct larger trials to detect meaningful early effects of treatment, **iii.** the need for a trial to examine the pre-exposure prophylaxis and postexposure prophylaxis efficacy of HCQ (Khan and Butler, 2020).

A further search at Clinical trial for HCQ in Covid-19 as of February 16, 2021, we found a total of 271 trials (233 interventional and 37 observational), 52 trials have been terminated/withdrawn/suspended, and 57 studies are completed. Approximately 161 studies were found for HCQ with status recruiting, not yet recruiting, active but not recruiting or enrolling by invitation only. Among these 13 studies are in Phase 1, 87 in Phase 2, 113 in Phase 3, and 23 in Phase 4 (see Fig. 3). Further, only 7 studies were found to be published with results. The critical trials are compiled in Table 2. The table importantly covers CQ/HCQ, their study design, and the last updated outcomes. Some significant outcomes from clinical trials are summarized in the subsequent section.

A RECOVERY collaborative randomized, controlled open-label platform trial explored the effect of HCQ in hospitalized patients with Covid-19. The trial explored randomization of patients to receive HCQ (n = 1561) and usual care (n = 3155) to visualise any significant effect on lowering death incidences at 28 days. The trial did not indicate any significant outcome in reducing death incidences in HCQ treated group (Group, 2020). In another clinical outcome, Self and group explored the efficacy of HCQ in hospitalized adult patients with Covid-19. During their study, they randomised patients to receive HCQ (n = 242) in comparison to placebo (n = 237). HCQ was dosed at 400 mg b.i.d with a total of two doses followed by 200 mg b.i.d with a total of 8 doses. The results disclosed no significant improvement in clinical outcome for patients with Covid-19 with symptoms of ARDS treated with HCQ as compared with placebo (Self et al., 2020). Another outcome of a randomized clinical trial by Rajasingham et al. explored the utility of HCQ in prophylaxis (pre-exposure) of Covid-19 in 1483 health workers at doses of 400 mg once or twice per week for 12 weeks. The study
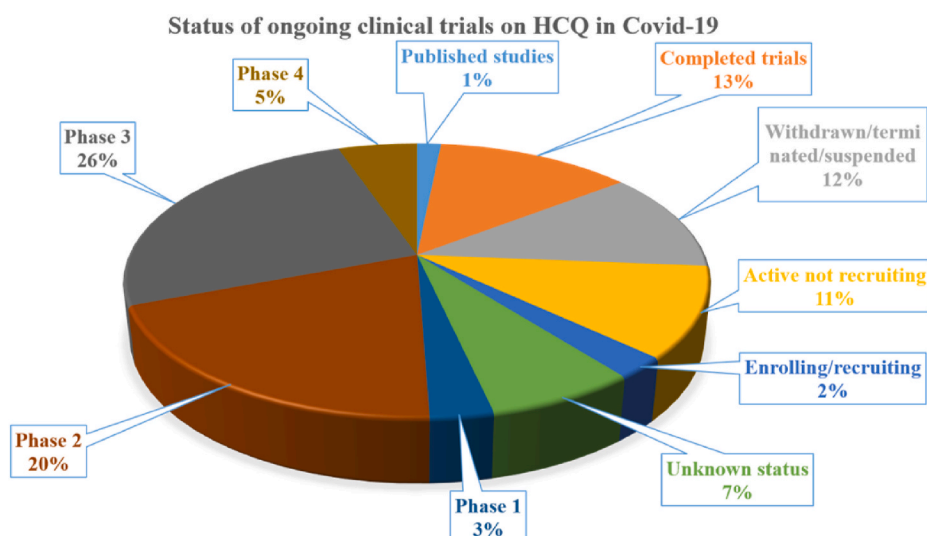
Fig. 3. Status of the clinical trial on HCQ (Data is as per https://clinicaltrials.gov/, assessed on February 16, 2021).

disclosed no significant outcome of HCQ in prophylaxis among healthcare workers (Rajasingham et al., 2020). In a similar attempt, randomized trial published by Barnabas and group concluded no meaningful clinical effect of HCQ for postexposure prophylaxis to prevent SARS-CoV-2 infection. The conclusion was made on the basis of outcomes between control (422 participants) and HCQ treated (407 participants) groups. The study highlighted no difference in improvements between the selected groups; however, the frequency of adverse events was higher in case HCQ treated group. HCQ was dosed at 400 mg per day for 3 days, followed by 200 mg per day for the next 11 days, the control group was dosed with ascorbic acid (Barnabas et al., 2020). Another cluster-randomized trial report published by Oriol and group explored the efficacy of HCQ in postexposure cases for prevention of Covid-19. The group disclosed a similar outcome as found by Barnabas and group. The study disclosed no efficacy of HCQ on postexposure therapy when dosed at 800 mg for day 1, followed by 400 mg once for next six days for healthy persons exposed to a patient with Covid-19 positive status. The conclusion was drawn out of the randomized trial conducted on healthy contacts of Covid-19 patients that were subjected to HCQ treatment (1116 participants) in comparison to control intervention or usual care (1198 participants). The highlights of the study were, HCQ did not lower the incidence to acquire Covid-19 in the exposed healthy participants, and at the time the group reported more incidences of adverse events as compared to control group (Mitja et al., 2021). Another study by Cavalcanti et al. explored the efficacy of HCQ in combination with azithromycin or alone in mild to moderate cases of Covid-19. They disclosed the outcome from their multicentre, randomized, open-label, three-group, controlled trial on 667 patients. However, the outcomes of the study were in disfavour of using HCQ alone or in combination in hospitalized patients with mild or moderate symptoms. The group established no correlation of HCQ efficacy with clinical status at 15 days in comparison with standard care (Cavalcanti et al., 2020). Further reports by Qaseem and group on the clinical use of CQ/HCQ alone or in combination with azithromycin suggested no evidence of effectiveness based on the results of three RCTs (Qaseem et al., 2020). A systematic review and meta-analysis published by Fiolet and group explored the evidence of HCQ use alone or in combination with azithromycin on mortality in Covid-19 patients (Fiolet et al., 2021). The conclusion by the group was derived on the basis of 25 observational studies, 3 randomized clinical trials and 1 non-randomized study (interventional). The important findings deduced were, **a.** HCQ is not associated with reduced mortality, which in contrast was increased when taken in combination with azithromycin; **b.** high heterogeneity

was observed among reported studies due to variable and unadjusted dosing; **c.** supports recommendation of NIH, that disfavour the HCQ use alone or in combination; **d.** suggests no need to explore HCQ alone or in combination for proving its efficacy in Covid-19. Further, the commentary published by April Jorge commented on the possibility of HCQ use to prevent Covid-19 although after the failure of multiple high-quality studies conducted on establishing HCQ efficacy in Covid-19 (Jorge, 2021). Jorge put forth the study by Rentsch and group, which explored the use of HCQ in preventing mortality in Covid-19 patients. The study focused on recruiting patients (n = 30569) with rheumatoid arthritis already using HCQ from last six months before the start of Covid-19 pandemic in England. The comparison was made with 164068 patients with rheumatoid arthritis using alternative medicines other than HCQ. The outcome of this highlighted no statistical differences in Covid-19 associated mortality in both the groups along with underlying no differences in non-Covid-19 mortality that was thought to be linked with HCQ use. This study was deemed important since it established the potential role of HCQ in the treatment of rheumatic and lupus disease. The study also subsided its use in the prophylaxis or treatment of Covid-19 at the same time and also discarded the possibility of HCQ in inducing adverse effects or any reduction in mortality among severe Covid-19 patients. In a similar line, Hernandez and group published three updates on the use of HCQ and CQ on various shreds of evidence published on their prophylaxis or use in treatment. Their first report suggested the high risk of bias and insufficient evidence to support the effectiveness or safety of CQ/HCQ in Covid-19. The group also focused on the need for RECOVERY, SOLIDARITY, and ORCHID trials for more conclusive results (Hernandez et al., 2020c). The second report was more focused on evidence provided by RECOVERY trial, which again failed to demonstrate the compelling evidence. Further, SOLIDARITY and ORCHID trials also failed to establish required results and were discontinued prematurely (Hernandez et al., 2020a). The third report also ended in the same conclusion that HCQ/CQ are ineffective for hospital patients use; however, the study underlined the evidence for outpatient use of HCQ (Hernandez et al., 2020b).

In the significant randomized trials conducted so far, no major significance was found. However, observational studies proved some sort of beneficial effect of HCQ alone or in combination. Further, on June 4, 2020, WHO, based on the evidence provided by MHRA that HCQ has no beneficial effect on hospitalized patients, stopped enrolling participants into the HCQ arm of the RECOVERY trial.

Moreover, few other drugs in addition to CQ/HCQ have been explored for their antiviral potential in Covid-19. The important ones

Table 2

Status of some selected trials studying the efficacy and safety of CQ/HCQ in patients with Covid-19.

Author/NCT	Type of study	Group (n)	Outcome
NCT04261517 (Chen et al., 2020a)	**A randomized study** on HCQ	30, Age 18 and above, all sex	No effect on viral clearance at day 7
Tang and group (Tang et al., 2020)	**A randomized controlled study** on HCQ (Recovery trial)	4674 patients, all sex	Mortality (28 patients) with no significant effect on hospital stay
Tang and group (Tang et al., 2020)	**A randomized study** on HCQ	150 patients, all sex	Insignificant, viral clearance by day 28 was 85.4% as compared to other interventions (81.3%)
Chen and group (Chen et al., 2020c)	**A randomized study** on HCQ	62 patients, all sex	Enhanced time for clinical recovery, improved cough remission time
Boulware and group (Boulware et al., 2020)	**Randomized study** (Prophylactic use) on HCQ	821 asymptomatic volunteers and health workers with high-risk exposure	Marginal difference in post-exposure incidence (11.8% with HCQ vs 14.3% with control interventions)
Magagnoli and group (Magagnoli et al., 2020)	**Non-randomized study** on HCQ alone or in combination with Azithromycin	368 patients, all sex	Mortality within the HCQ group (27%), HCQ + Azithromycin combined group (22.1%), control group (11.4%); Ventilation condition was not improved either.
Gao and group (Gao et al., 2020)	**Observational study** on CQ and HCQ	100 patients, all sex	Shortened disease outcome and inhibited associated pneumonia
Gautret and group (Gautret et al., 2020b)	**Observational study** on HCQ alone or in combination with Azithromycin	42 patients, all sex	Combination improved viral clearance at day 6 (70% (controlled intervention: 12.5%)
Gautret and group (Gautret et al., 2020a)	**Observational study** on HCQ alone or in combination with Azithromycin	80 patients, all sex	Viral clearance was observed at day 7 (83%) shortened the hospital stay
Molina and group (Molina et al., 2020)	**Observational study** on HCQ in combination with Azithromycin	11 patients, all sex	Viral clearance was recorded at day 6 (20%)
Mahevas and group (Mahevas et al., 2020)	**Observational study** on HCQ	181 patients, all sex	Insignificant outcomes, no improvements in deaths and associated pneumonia
Chatterjee and group (Chatterjee et al., 2020)	**Observational study** on HCQ	Healthcare workers	A significant decline in chances of getting infected (95%)
Borba and group (Borba et al., 2020)	**Interim analysis** on HCQ	400 patients, all sex	Higher mortality was observed with increase in dose regimen
NCT04435808	**The interventional study,** for determination of efficacy for HCQ/CQ in health workers with a high risk of Covid-19	1 enrolled, all sex, above 18 years	Completed, study terminated (Stopped for futility by DSMB)
NCT04329923	**The interventional study,** PATCH trial	173 enrolled, all sex, above 18 years	Terminated, Reasons: (Cohort 1: slow accrual Cohort 2: Other studies showed no benefit Cohort 3: Study met pre-specified futility analysis at planned second interim analysis)
NCT04491994	**The interventional study,** for the efficacy of HCQ in Covid-19 progression	540 enrolled, all sex, above 18 years	Completed, no results disclosed yet
NCT04434144	**Observational study** to compare the efficacy of Ivermectin and HCQ on the COVID19 Patients in Bangladesh	116, Age 16–80 years, all sex	Completed, no results disclosed yet
NCT04376814	**Non-randomized study** to examine the efficacy of Favipiravir Plus HCQ and Lopinavir/Ritonavir Plus HCQ in COVID-19	40, Age 16–100 years, all sex	Completed, no results disclosed yet
NCT04334967	**Randomized study** to examine the effect of HCQ in patients with newly diagnosed covid-19 compared to standard of care	13 patients, all sex	Suspended
NCT04333654	**Randomized study** showcasing the effect of HCQ in outpatient adults with COVID-19	210 patients, all sex	Suspended
NCT04341727	**Randomized study** to analyze the effect of HCQ and HCQ-Azithromycin combination in the treatment of Covid-19	500 patients, all sex	Suspended
NCT04348474	**Interventional study** to evaluate the efficacy and safety of HCQ and azithromycin for the treatment of ambulatory patients with mild covid-19	200	Suspended
NCT04369742	**Randomized study** to analyze the effect of treatment of Covid-19 with HCQ	626	Suspended
NCT04329572	**Interventional study** to analyze the efficacy and safety of HCQ and azithromycin for the treatment of hospitalized patients with moderate to severe covid-19	400	Suspended
NCT04371926	**Randomized study** to understand the prophylactic benefit of HCQ in COVID-19 cases with mild to moderate symptoms and in healthcare workers with high exposure risk	0	Withdrawn
NCT04347512	**Randomized study** to examine the efficacy of the HCQ and azithromycin combination in the prevention of covid-19	0	Withdrawn
NCT04354441	**Non-Randomized study** to understand the effect of HCQ in COVID-19 positive pregnant women	0	Withdrawn
NCT04350450	**Non-Randomized study** to examine the HCQ treatment of healthcare workers with covid19 illness at Montefiore	0	Withdrawn
NCT04307693	**Randomized study** for comparison of Lopinavir/Ritonavir or HCQ in patients with mild Covid-19	65	Terminated
NCT04362332	**Randomized study** to see the effect of CQ/HCQ for supportive care in patients admitted with moderate to severe Covid-19	25	Terminated
NCT04345861	**Randomized study** to analyze the effect of HCQ plus Azithromycin Vs. HCQ for COVID-19	7	Terminated

are compiled in Table 3 with brief details about indication and current ongoing trials.

6. Author's opinion in predicting the use of HCQ in Covid-19: Conclusion

As per evidence (latest of February 16, 2021), 4396 publications (distributed as per Fig. 4A) have come in a year for the critical search of "Hydroxychloroquine AND Covid-19". The majority of papers have been published by United States (1247), Italy (631), India (493), Spain (307), France (287), United Kingdom (259), and China (230) (Fig. 4B). The studies are supported chiefly by the National Institutes of Health (116), National Natural Science Foundation of China (58) Gilead Sciences (35), National Institute of Allergy and Infectious Diseases (28) and Novartis (28). The research and published research papers presented a mixed judgment of using HCQ alone or in combination for treatment or prophylactic management of Covid-19. However, WHO called off all the ongoing trials and revoked the use of HCQ for the treatment of Covid-19 disease. Much controversies and debate still exist on the use or disuse of HCQ for treatment or prevention of Covid-19. As our knowledge goes, and we reviewed the pandemic literature at greater depth (Poduri et al., 2020), we grouped HCQ use at four various stages. The stages include **i.** asymptomatic patients; **ii.** patient having mild symptoms; **iii.** patients with extreme symptoms, and finally, **iv.** high-risk population, which includes health workers or in close vicinity with Covid-19 patients.

The majority of Covid-19 cases are undetectable due to no significant physical symptoms, although this carries the SARS-CoV-2 virus, and thus, treatment of this undetected population with CQ/HCQ may lead to suppression of innate and adaptive immunity. This may inversely lead to the spread of the virus to a larger population. In the second case, although CQ/HCQ has produced sound *in vitro* results but has failed to transform the efficacy to the *in vivo* results, therefore the use of CQ/HCQ also gets dampen at this stage. Although, the reported studies have a huge disparity in outcomes. Reported *in vitro* studies have a fluctuation of almost 24 folds in values of EC_{50}, which is statistically insignificant and further hampered by the use of numerous cell lines, which makes the outcome uncertain. The difference urges a vital requirement to have an optimal target in *in vitro* systems to develop concrete results pertaining to the efficacy of HCQ. Moreover, these *in vitro* studies have not significantly transformed for predicting HCQ doses for human use in Covid-19. Further, the outcome is also worsened by poorly understood pharmacokinetics parameters of HCQ in Covid-19 patients. There is a vital requirement of correlation between HCQ dose with its pharmacokinetics and its monitoring to ensure the safety and efficacy in the patients. As far as toxicity with HCQ is concerned, reported toxicities of ocular, gastrointestinal, and cardiac are associated with long-term use and would not be of much relevance in treating Covid-19 disease, which is of concise duration (Mavrikakis et al., 1996; Melles and Marmor, 2014). Further, to overcome associated cardiac toxicities (cardiomyopathy, QT prolongation, torsade's de Pointes and ventricular arrhythmia), dose-response calibration along with baseline and periodic QT monitoring may be vital in considering future dose regimes. Further, no immediate reports concerning cardiac toxicities over short-term use are reported (Joyce et al., 2013). Further few reports concerning cardiac toxicity were due to combinational use of HCQ with macrolides in Covid-19 or with prevailing comorbid states (Khuroo et al., 2020). Thus there are essential research gaps that hamper the HCQ use at this stage.

Also, assessing the cause of organ damage via the use of CQ/HCQ is a complex diagnostic task. This is major because disease like rheumatoid arthritis and systemic lupus erythematosus in which CQ/HCQ is prescribed most often involves a cardiovascular system, and the associated symptoms are frequently nonspecific enough to initiate a diagnosis. Moreover, as diagnostic tools are the concerned determination of CQ/HCQ blood level is not accurate by them plausibly due to complex pharmacokinetics of these drug inter-individual variation in metabolism. Still, there lie few diagnostic tools, used to measure the impact of

these drugs on cardiotoxicity. The important one includes cardiac imaging majorly via magnetic resonance imaging (T1 mapping), endomyocardial biopsy, echocardiography, histological assessment using ultra electron microscopy and differential diagnostic. (Chatre et al., 2018; Tönnesmann et al., 2013). Among these, echocardiography has emerged to found utility in the diagnosis of acute cardiac complications and treatment monitoring in Covid-19. Echocardiography deploys ultrasound waves to create an assessment of heart, its size, thickness, wall movement, working of heart valves, and conditions of regurgitation and stenosis. It is divided into various subtypes depending on utility, important one includes, Transthoracic echocardiography; Transesophageal echocardiography; Stress echocardiography; 3D-echocardiography and Fetal echocardiography. Among all transthoracic echocardiography is widely used in the detection of cardiac toxicities induced by therapeutic drugs. It allows assessment of myocardial strain and thus supports the diagnosis of myocarditis. The echocardiography thus may reveal early detection of myocardial dysfunction and thus allow possible treatment discontinuation before severe toxicity precipitates (Vrettou et al., 2020).

At last, the third category confines to the inflammation in the form of cytokine storm and severe ARDS take over; therefore, using CQ/HCQ treatment at this stage would be beneficial owing to the reports it can overcome cytokine storm and improves ARDS. Though clinical evidence disfavors the use of this anti-malarial drug at this point, substantial shreds of evidence from planned randomized trial taking along its pharmacokinetics parameters are the need of hour before drawing the final conclusion.

Finally, as it has been exemplary, saying "*prevention is better than cure,*" so the use of CQ/HCQ is the rationale to prevent the entry of viruses in patients with high risk, including health workers. Many studies are currently ongoing to explore the beneficial effect of HCQ on this class. The major hurdle of pharmacokinetics is being tried to resolve using the nasal route *via* inhalation or spray formulation (Kavanagh et al., 2020). When talking about efforts made to deduce the doses of HCQ for Covid-19 patients, there include only rare studies that include two clinical evidences (Al-Kofahi et al., 2020; Painvin et al., 2020) and few model-based predictions (Garcia-Cremades et al., 2020; Thémans et al., 2020; Yao et al., 2020). The model developed significantly predicts the optimal doses for the viral decline and QT prolongation. The model developed by Garcia et al. considered the reported EC_{50} values ranging from 0.72 to 17.31 μM; during their stimulation studies, the group found extrapolated IC_{50} to be 4.7–7.5 μM for optimal and efficacious inhibition of viral replication. The group also predicted that a concentration above 7.5 μM could cause toxicity. Further, based on the PK model group also disclosed, the HCQ regimen to be 400–600 mg bid for five days for decreasing the viral load with above 600 mg dose for the same duration could lead to QT prolongation. Further following the *in vitro* results of Yao et al. and HCQ superiority over CQ developed a PBPK (Physiologically based pharmacokinetics) model. The model was used to predict the HCQ doses in the lung fluid stimulation system involving experimental data based on human pharmacokinetics and data generated from HCQ studies on rat lung penetration. Based on the RLTEC score (ratios of estimated free lung tissue trough concentration to EC50), the group predicted a 400 mg bid dose of HCQ for day 1, followed by 200 mg bid for the next 4 days. Themans et al. during their study, explored the comparative effect of numerous dosing protocols reported for HCQ in Covid-19 and validated using independent data in lupus erythematosus patients. Based on Monte Carlo simulations, the group predicted dosing of 800 mg for day 1, followed by 600 mg for the next 5 days. The group decided this regimen for 80% population with the need for dose optimization in comorbid and other disease conditions of the body, thus concerning HCQ dosing is an unmet need in Covid-19.

Apart from treatment based *in silico* models, few models for predicting doses for prophylactic use of HCQ have also been explored. A model developed by Al-Kofahi and group developed a model using inputs from reported plasma concentration of HCQ in malaria patients and

Table 3
Comparative status of other antivirals explored in Covid-19.

Drug name	Target in SARS-CoV-2	Mechanism in Covid-19	Clinical trials[a]	Recommendation as per NIH
Remdesivir (only approved drug in Covid-19)	Viral RNA-dependent RNA polymerase (RdRp)	Block RdRp action allowing faulty proofreading by viral exoribonuclease	86	200 mg via IV route for day 1, followed by 100 mg IV for 4 days or until hospital discharge, whichever comes first
Favipiravir	RNA polymerase	Inhibit RNA polymerase involved in the RNA transcription	48	Not recommended for treatment except for clinical trial
Umifenovir	hemagglutinin protein	Inhibits recognition of S protein and its membrane fusion with ACE2	11	Not recommended for treatment except for clinical trial
Niclosamide	Not known	Target the viral reservoir in the gut region and consequently decreases viral load	13	Not recommended for treatment except for clinical trial
Ivermectin	importin $\alpha/\beta 1$	Inhibits integrase protein nuclear import via importin $\alpha/\beta 1$	59	Not recommended for treatment except for clinical trial
Combination of Lopinavir/ritonavir	Viral protease 3CLpro	Inhibiting viral protease 3CLpro and consequently blocks virion assembly	55	Not recommended for treatment except for clinical trial

[a] Data is retrieved from https://www.clinicaltrials.gov/using keywords "drug name" AND "Covid-19".
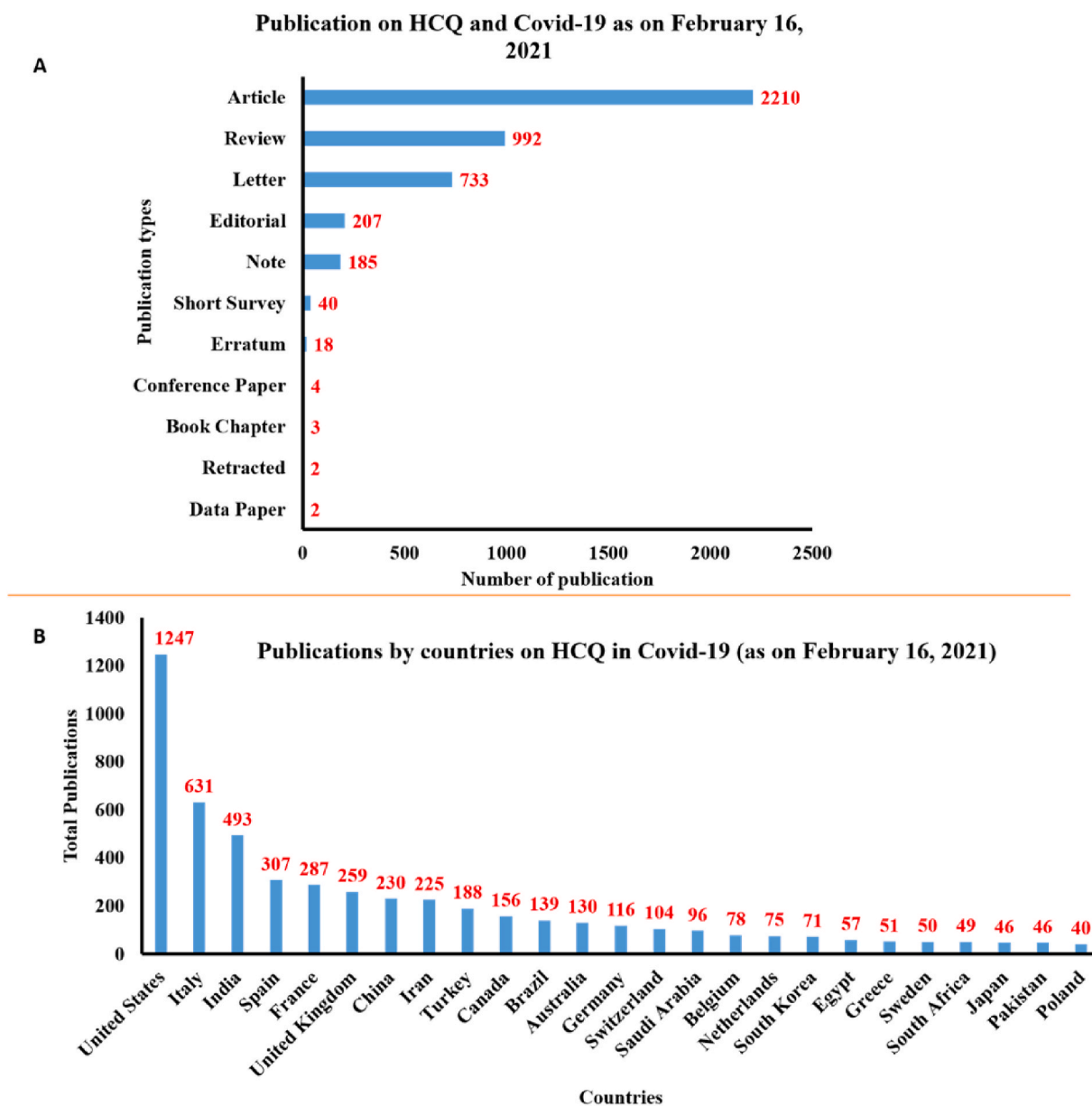

Fig. 4. A. Bar-graph represents total number of publication for HCQ in Covid-19, **B.** Bar-graph represents total publications by countries (for clarity, only those countries that published 40 or more articles on the theme are included). The data was curated as per the Scopus database, assessed on February 16, 2021.

healthy volunteers to predict plausible pre and post-exposure HCQ drug dose. The group exploited slightly above doses of *in vitro* reported against SARS-CoV-2 (0.72–17.31 μM) to account for loss due to plasma protein binding of the drug. The analysis revealed for prophylaxis 200 mg tid for 6 days with no loading dose, however for post-exposure study suggested a loading dose of 800 mg followed by 600 mg daily for 3 days (Al-Kofahi et al., 2020). However, the model predicted studies were not corroborated biologically, which thus hampers their authenticity and did not disclose much about the proven target(s) if any.

It is suggested that the timing of administration and the PK properties of HCQ are a significant influencer on the outcome of the treatment of disease. In the current compilation, we expedite through various literature and available evidence for a plausible role of CQ/HCQ in Covid-19. The critical gaps identified during this work include **i.** *In vitro* and *in vivo,* assays need much more calibration to adequately and precisely define the inhibitory potential of HCQ; **ii.** Development of optimal pharmacokinetics models, both theoretically and biologically, to define the therapeutic dosing system to lower the viral load without provoking side effects or doses regimen for prophylactic use; **iii.** experiments to assess the concentration of HCQ in lungs, along with V_d in the various compartment during treatment duration in human model affected with Covid-19 are required; and **iv.** more experimentation exploring the combination role of HCQ with other important repurposed molecules in Covid-19 to decrease the viral load and improve the efficacy further via synergistic mechanism; **v.** thorough and robust experiments to explore HCQ utility in prophylaxis versus mild versus moderate versus severe disease. Further, the critical gap which we believe comes is from the clinical trials conducted concerning the need for good well defined unbiased randomized clinical trial to corroborate the same findings. Although the things went very well during *in vitro* studies and in the initial phases of the pandemic, which was shortly dampened by clouds of unefficacious results detected in a handful of clinical trials conducted. Some studies though suggested better outcomes for HCQ use in patients of Covid-19 or for its prophylactic use, and the studies still have some methodological limitations. The critical limitation in clinical evidence is **i.** high risk and biased studies, **ii.** no studies were done on critically ill patients with co-morbidities existing, **iii.** the period of treatment was concise, which again points towards the authenticity of trials, **iv.** dosing error was almost there with every trial, with dose ranging from 400 mg to 1200 mg within a span of 5–10 days, with no due consideration given to obese patients, paediatric population, pregnancy, and patients with other complications like diabetes, cancer, respiratory or cardiovascular disorders. The research/editorials by pioneer journals, including the New England Journal of Medicine and Lancet has also emphasized randomized controlled trials that should be well designed and adequately powered to prove the efficacy of HCQ. Further, we are in 14 months since the pandemic started, and there is indistinct evidence as to which drug regimen may work well. At the same time, Covid-19 has led to the death of 2,399,103 lives (https://covid19.who.int/; assessed on February 16, 2021) during the writing of this manuscript. The authors are, therefore, of the opinion that though viral clearance is foremost vital in Covid-19, still, the medium-long term trial outcomes should be forecasted before disapproving a proven therapeutic regime, HCQ. Thus, more emphasis needs to be put on the pharmacokinetics of HCQ along with monitoring the doses supported by sound evidence from robust randomized clinical trials to answer the efficacy of HCQ for treatment or chemo-prophylactic use against Covid-19. Further, in lights of discussed criticalities in the current manuscript, numerous investigations are ongoing to further investigate the effects of HCQ in Covid-19, which is a great and healthy sign. The crucial questions that need to be unravelled include **i.** Where to fit HCQ is current regimen, whether it should be should for prophylactic use, under mild or moderate or severe conditions of Covid-19? **ii.** What could be the alternatives proven regimen that could drastically synergize the effect of HCQ in Covid-19?

Author contributions

The manuscript has been prepared and drafted with mutual understanding and contributions of all the authors.

Credit authorship contribution statement

Gaurav Joshi: Ideation, Writing - original draft, review & editing. **Shikha Thakur:** Writing - original draft. **Mayank:** Writing – original draft. **Ramarao Poduri:** Ideation, Curation and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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