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Review Article

Hydroxychloroquine for COVID-19: A review and a debate based on available clinical trials/case studies

Kirtikumar C. Badgular^{1*}, Ashish B. Badgular², Vikrant P. Patil³, Dipak V. Dhangar⁴¹ Assistant Professor, Department of Chemistry, SIES College of Arts Science and Commerce, Sion West, Mumbai 400022, India, Contact no: (+91) 9860180002² Ophthalmologist, Municipal Eye Hospital, Kamathipura, Mumbai 400008, Maharashtra, India³ Consulting Physician, Dwarka Clinic Amalner 425401, Maharashtra, India⁴ DNB Respiratory medicine resident, Bombay hospital and Medical Research Centre, Mumbai 400020, Maharashtra, India

ABSTRACT

Hydroxychloroquine (HCQ) as a drug grabbed serious attention of whole world in dealing with COVID-19 pandemic. Recently some in-vitro and in-vivo study showing possible inhibition of SARS-CoV-2 by use of HCQ. However at the same time, some case studies showing NO clinical benefit/ poor clinical outcome with substantial detrimental adverse effects by use of HCQ in treatment of coronavirus disease-2019. Thus, the HCQ use (in COVID-19 treatment) is of current international interest, although a consensus has not yet been reached. More evidences are still required to prove efficacy of HCQ against COVID-19. In view of this, the present review highlights the current ongoing research related to use of HCQ in treatment coronavirus disease-2019. The present review will discuss the possible anti-viral mechanism of HCQ, prophylaxis strategy and effect of HCQ against SARS-CoV-2 virus in-vitro study. Further this review also summarizes and debates all available clinical trials/ case studies of HCQ use against COVID-19 (with clinical outcome). Finally possible detrimental adverse effects are also discussed considering the public health and pharmacovigilance concern.

Keywords: Potency of hydroxychloroquine; COVID-19; Coronavirus disease-2019; Clinical trials; SARS-CoV-2; Adverse effects of hydroxychloroquine; Pharmacovigilance concern

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*Address for Correspondence:

Dr. Kirtikumar Chandulal Badgular, Ph.D., Assistant Professor, Department of Chemistry, SIES College of Arts Science and Commerce, Sion West, Mumbai 400022, India

1. Introduction:

Year 2020 started with the novel coronavirus 2019 disease (COVID-19) which has been declared a pandemic [1]. It is third extremely pathogenic and contagious coronavirus after endemic SARS-CoV and MERS-CoV appeared in humans [2]. The exact origin, transmission, mechanisms of SARS-CoV-2 is not confirmed and clear until now, however, its genome sequence is closely correlated (76-80 %) with the SARS-CoV coronavirus [2]. As of now (25th April 2020), approximately 29,01,305 patients have been confirmed to have COVID-19 infection globally (212 countries) with 2,00,082 fatalities [3]. Development of potential therapy is urgently required in order to prevent pandemic COVID-19. On 30th March 2020, Food and Drug Administration of USA issued an Emergency Use Authorization of HCQ and chloroquine (CQ) for treatment of certain COVID-19 patients [4].

A controversial anti-malaria drug being explored and used in random clinical trials and clinical case studies around the world against potential COVID-19 in order to get relieve from symptoms, or as a preventative measure to stop people being get infected with COVID-19 [5-10]. Some reports showing potential use of the HCQ in treatment of coronavirus disease-2019 (with minimum side effects) [5,6,9]. At the same time some reports are NOT at all supporting potency of HCQ for COVID-19 [7,8,10], conversely they reported increase of adverse events by use of HCQ in COVID-19 patient [10]. Thus, there are several toxic effects of HCQ which are needed to address considering prophylaxis use in view of pharmacovigilance concern [7,8,10]. It may be toxic enough that many people may extensively suffer or may even die due to cardiac problem during the treatment [10]. Hence a very careful analysis is still needed for the use of HCQ in treatment of coronavirus disease-2019 [7,8,10]. Thus, the debate for use

of the HCQ against COVID-19 is of great attention which needs an urgent consideration in order to understand the actual potency of HCQ against COVID-19 [5-10].

The use of the HCQ medicine in treatment of coronavirus disease-2019 is of current international interest, although a consensus has not yet been reached which may ask for more powerful evidences. In view of this, the present review is done to highlight (i) the antiviral use of HCQ against various viruses, (ii) possible mode of action of HCQ against SARS-CoV-2, (iii) in vitro-inhibition of SARS-CoV-2 virus by HCQ, (iv) conflicts in-vivo clinical trials/case studies explaining the therapeutic role of HCQ in coronavirus disease-2019 patient, (v) prophylactic use of HCQ (vi) side effects and pharmacovigilance concern of use of HCQ.

2. 4-Aminoquinolines and their anti-viral therapeutics applications:

Hydroxychloroquine and chloroquine are 4-aminoquinoline derivatives. The change in *N*-diethyl side chain of CQ by *N*-ethyl hydroxyl group in HCQ offers more solubility. Further, HCQ showed significant bioavailability and immunomodulatory effects by interfering interleukin-6 synthesis (IL-6) which has essential role in chronic inflammation and auto-immunity [11-13]. Furthermore, HCQ produces less toxic metabolites, less adverse effects and hence considered safer drug than CQ [11-14]. Besides this, HCQ also displayed anti-viral activity by stimulating the pathway of IFN- β via phosphorylation [11,12]. HCQ is primarily an anti-malarial drug which is also used in autoimmune diseases [11-13], sarcoidosis [14], rheumatoid arthritis [15], alopecia areata [16], lupus erythematosus [17], anti-thrombotic [18] and antineoplastic treatment [19]. Moreover, HCQ shows potential antiviral activity and therapeutic use against Ebola [20], Marburg [20], Zika [21], HIV-1 [22], Dengue [23], Chikungunya [24], Hepatitis C [25], MERS-CoV [26] and SARS-CoV [27] virus treatment.

Various pathways for mechanism of action of HCQ are explained against virus [20-27]. Haque et al., [20] proposed that, HCQ deposition in the endosome increases the pH and causes inactivation of lysosomal enzymes as well as receptors which inhibits entry of virus. Kumar et al. [21] reported that, HCQ activates the innate immune signal pathways and promote the production of reactive oxygen species which inhibit Zika virus cycle. Romanelli et al. [22] mentioned that, HCQ may block hydrolysis of polypeptide product and HIV virus replication [22]. Further increasing endosomal pH changes the activity of hydrolases enzyme which inhibits glycoprotein production [22]. Wang et al., [23] found that, HCQ activates innate immune signal pathways and interferes mitochondrial viral signalling [23]. Further, HCQ induces production of cellular reactive oxygen which imparts in host defence system [23]. Pandya et al., [24] reported that, mechanism of HCQ action against Chikungunya virus is still unclear; however, it is related to anti-inflammatory response. Chandramohan et al., [25] mentioned that, HCQ is responsible for blocking of endocytosis mechanism required for hepatitis C virus entry. Increase in lysosomal pH causes inactivation of lysosomal enzymes which prevent further viral replication. Dyal et al

[26] and Yao et al., [27] proposed that, HCQ affects entry of virus, increases the pH of endosome. Further, it knocks down the endocytosis process and viral replication phenomenon in corona viruses. Thus HCQ plays an important role against various viral infections and hence similar kind of mechanistic role may be expected in case of action of HCQ against COVID-19 virus which may needs strong evidences to prove it.

3. Effects of HCQ on the immune system and COVID-19:

In case of COVID-19, the lung injury is more obvious in critical patients that are directly linked with a cytokine storm. The pro-inflammatory cytokine storm with a severe illness affected the prognosis. Various IL-6 antibody blockers, convalescent plasma therapies have been applied to offset the cytokine storm. Hence it is predicted that, HCQ may be used against COVID-19, considering its antiviral [20-27] and immunomodulatory effects [11-13]. The excessive productions of cytokines lead to obstruct innate immune response [11-13]. The HCQ regulates or modulates the production of different cytokines (like IL-6, IL-1, IL-2) which are involved in the cytokine storm [11]. The HCQ also displayed anti-viral activity by stimulating the pathway of IFN- β via phosphorylation [11,12]. Further, HCQ endorse post-translational adaptation of signalling proteins to modulate innate immune responses and thus it is postulated that, antiviral and immuno-modulatory effects of HCQ may synergistically combat the COVID-19 infection [11,12,28]. Besides this, HCQ considerably induces cellular production of reactive oxygen radical/species, which shows a significant role in host defence mechanism [11,28]. Thus, it is possible that, HCQ may be displaying an emerging role in regulating and alleviating the host innate immunity against COVID-19, however it requires further investigation to confirm the actual role of HCQ against COVID-19 considering innate immunity factor [11,12,28].

4. In vitro inhibition of SARS-CoV-2 by HCQ and CQ

Recently some reports are available which demonstrated in-vitro [27,29] (Table 1, entries 1-8) and in-vivo [5-10] inhibition of SARS-CoV-2 virus by use of HCQ. Liu et al., [29] have studied antiviral effects of HCQ and CQ against the SARS-CoV-2 virus infection at various multiplicities of infection (MOI). They measured in vitro cytotoxicity of HCQ and CQ in African monkey kidney Vero-E6-cells [29]. They reported 249 and 273 μ M CC_{50} (50% cytotoxic concentration) values for HCQ and CQ respectively (Table 1, entries 1,2). Further, EC_{50} (50 % effective maximal concentration) values was found to be slightly higher for HCQ than CQ which indicating lesser potency of HCQ over CQ (Table 1, entries 1-4) [29]. Considering the SI (selectivity index), CQ is found to be more potent than HCQ (Table 1, entry 1-4). However, author proposed the use of HCQ considering the safety and toxicity concern. In context to this, Yao et al., [27] have reported lesser EC_{50} value for HCQ (6.14 and 0.72 μ M) than CQ (23.9 and 5.47 μ M) at 24 and 48 hours respectively which indicating that HCQ may be a more potent and safer drug than CQ to treat COVID-19 by considering in-vitro antiviral activity (Table 1, entry 5-8).

Table 1: In vitro inhibition of SARS-CoV-2 virus by hydroxychloroquine and chloroquine

Entry	Drug	EC ₅₀	CC ₅₀	SI	IC ₅₀	Time	Ref.
1.	Chloroquine (0.01 MOI)	2.71	273	100.81	NM	48 h	29
2.	Hydroxychloroquine (0.01 MOI)	4.51	249	55.32	NM	48 h	29
3.	Chloroquine (0.02 MOI)	3.81	273	71.71	NM	48 h	29
4.	Hydroxychloroquine (0.02 MOI)	4.06	249	61.45	NM	48 h	29
5.	Hydroxychloroquine	6.14	NM	NM	NM	24 h	27
6.	Hydroxychloroquine	0.72	NM	NM	NM	48 h	27
7.	Chloroquine	23.9	NM	NM	NM	24 h	27
8.	Chloroquine	5.47	NM	NM	NM	48 h	27

Unit for EC₅₀ and CC₅₀: μ M

Liu et al., [29] have confirmed these in vitro antiviral activity results by immunofluorescence microscopy analysis study which suggested that, HCQ and CQ causes blocking of transport of SARS-CoV-2 virus and its genome from colocalization with early endosomes. Further, they assumed blocking of the endocytosis phenomenon due to endosome maturation attributed to change in endosome pH [29]. Thus both authors [27,29], concluded HCQ as a safer and less toxic drug to treat COVID-19 on the basis of in vitro analysis study.

5. HCQ: Possible mechanism of action against COVID-19:

Possible mechanism is proposed on the basis of initial assessment of HCQ as a drug against SARS-CoV-2 virus [27,29]. It is postulated that, HCQ functions in three different ways against the SARS-CoV-2 virus [29-33]. Angiotensin-converting enzyme-2 (ACE-2) which was considered as a possible binding receptor for SARS-CoV is also known as a possible receptor for SARS-CoV-2 considering the amino acid homology [30]. It is recognized that, SARS-CoV-2 having 76 % amino acid homology to that of SARS-CoV [2,3]. Thus it may be possible that, SARS-CoV-2 virus follows the similar kind of mechanism like SARS-CoV virus to enter into the host-cell [2,3]. SARS-CoV-2 has spike protein which involves the two subunits namely S1 and S2 [2,3,30]. The first S1-subunit is showing binding ability towards receptor ACE-2 (in adipose tissues); whereas, second S2-subunit is involving in membrane fusion and insertion of RNA into the cell [27,29,30]. Various mechanisms have been postulated in the literature about the antiviral action of HCQ which affects the viral entry pathway by decreasing the glycosylation of ACE-2 [27,29,30]. Since, it is assumed that virus binds to ACE-2 receptor to enter inside the host cell.

HCQ is a weak base which can be accumulated within acidic organelles (endosome, Golgi vesicles and lysosomes) wherein actual pH is found to be low. After accumulation, the HCQ leads to affect acidic organelles as well as functioning of several acid hydrolase enzymes by increasing the pH of organelles [31]. Some viruses enter by endocytosis mechanism in which virus targets the lysosomes [31]. These lysosomes having low pH and by action of acid hydrolase enzymes viral particles are disrupted which liberates various viral contents (infections material, nucleic acid and enzymes) that required for viral replication [31]. The accumulated HCQ in acidic organelles has been found to hamper the process of endocytosis and subsequent virus replication by increasing the pH level of lysosomes. Thus, HCQ is assumed to affect the viral entry [31].

In some cases virus is transported to endosome and gets replicated in it [32]. These endosome are get ruptured by

low pH acidic intracellular lysosome enzymes and leads to relieve the viral contents and replicated virions [32]. It is believed that, accumulation of HCQ increases the pH level of endosome as well as Golgi network and interfere functioning of various important acid hydrolases enzymes that hamper synthesis of new protein modification and viral inside-outside entry mechanism [32]. Thus in conclusion, these HCQ changes the pH of cell organelles and inhibit the fusion process of virions, replication, glycosylation of viral proteins, endocytosis, virion transport and virion release [27,29,30-32].

6. In vivo inhibition of SARS-CoV-2:

Till date only 9 clinical trials/case study reports are available which indicating the use of HCQ in COVID-19. Some case studies are showing good outcome with the HCQ treatment while some case studies are reporting poor clinical outcome with substantial adverse effects. Chen et al. [5], reported open 1:1 controlled trial study in COVID-19 patients treated with the HCQ which indicated no significant effect of HCQ in treatment of COVID-19 patients compared to that of control group. 33.3 % patients from the HCQ group and 46.7 % patients from control group showed radiographic progression in follow-up examination (Table 2, entry 1). Throat swab for SARS-CoV-2 was found to be negative for 86.7% and 93.3% cases of HCQ group and control group respectively. Thus, they did not observe any specific distinction in between HCQ treatment and conventional treatment for COVID-19. The major limitation of this study is the smaller sample size and lower statistical significance.

A French group Gautret et al., [6] have reported effective use of hydroxychloroquine + azithromycin (HCQ + AZT) in treatment of COVID-19 which cured 100 % patients on sixth day whereas, 57 % patients were cured virologically by only HCQ treatment on sixth day (Table 2, entry 2). In comparison to this, 12.5% patients were cured virologically in control group on sixth day. The total duration of treatment/medication was ten days. In their report, no specific adverse effects (of use of HCQ +AZT) are mentioned except one death. This study has several limitations such as small sample size, limited follow-up, exclusion of patients during treatment and specific age group. However, another French group Molina et al., [7] reported no clinical benefit and poor clinical outcome with the synergetic treatment of AZT+HCQ in coronavirus disease-2019 patients (Table 2, entry 3). In their study, nasopharyngeal swab for SARS CoV-2 virus were found to be positive in 80% patients on fifth day. The major limitation of this report is the absence of control study. Further, they have not reported details of study and statistical significance, since it is a letter to editor.

Chen et al. [8], carried out 1:1 randomized clinical trials

which indicating faster clinical recovery, pneumonia absorption and cough reduction in HCQ treated group as compared to control group (Table 2, entry 4). They showed improvement in pneumonia in 80.6% of HCQ treated patients compared to 54.8% of control group. Standard treatment involves use of oxygen therapy, immunoglobulin, antiviral use, antibacterial use with or without corticosteroids. They proposed that, HCQ acted as a protector for COVID-19 patient due to its antiviral and immunomodulatory effects. Two patients of HCQ treated group showed adverse effects, thus adverse effects needs to be address carefully in the HCQ treated patients. Further in another report of 80 mildly infected patients, Gautret et al. [9] have confirmed potential use of HCQ + AZT therapy for COVID-19 (Table 2, entry 5). In this study, they reported death of one patient, and in whole study they performed ECG in order to understand the detrimental side effects of HCQ + AZT. On fifth day, 97.5% of patients showed negative virus culture from respiratory sample, however, the major limitation of this study is the absence of control group.

Notably, Chorin et al., [10] reported QT prolongation in number of patients due to treatment of HCQ and AZT in between 3-4 days. In this report, 30% of patients showed QT prolongation (> 40 ms), while 11% of patients showed QT increased (> 500 ms) which indicating the high risk arrhythmia (Table 2, entry 6). They reported death of four patients in a set of 84 patients. Tang et al., [33] mentioned use of HCQ against COVID-19 patients. They found that, medication of HCQ results in more alleviation of clinical symptoms than standard of care treatment (Table 2, entry 7). Specifically they mentioned that, viral response not increases but it accelerates the alleviation of clinical symptoms and recovery of lymphopenia. In final conclusion, no difference was observed in the main outcome of the HCQ group and control group. Mahevas et al. [34], carried out routine data analysis of 181 patients which concluded non-supportive use of HCQ for COVID-19 in their study compared to control group (Table 2, entry 8). Further, they found modification in ECG in 9.5% patient treated with HCQ. They reported death of

2.8% and 4.6% patients in HCQ treated and control patient's group respectively. Moreover, 28.6 % and 24.2 % patients from HCQ and control group showed development of acute respiratory distress syndrome.

Magagnoli et al., [35] reported retrospective analysis of 368 patients which are divided into three categories such as HCQ group, HCQ+AZT group and control group (Table 2, entry 9). They observed higher death ratio in HCQ treated group. They reported the rate of death as 27.8%, 22.1%, and 11.4%, in the HCQ, HCQ+AZT and control group respectively. Further they mentioned that, specific precaution is required for the use of HCQ which may show severe adverse effects. Thus the maximum number of recovery/discharge was from control group and not from HCQ treated group.

Thus in conclusion, there are several dispute in use of HCQ for the treatment of COVID-19. Gautret et al., [6,9] and Chen et al., [8] supported the use of HCQ alone or in combination with antibiotic such as AZT. However, Chen et al., [5] Molina et al., [7] Mahaves et al., [34], Chorin et al., [10] and Magagnoli et al., [35] did not reported any significant effect/clinical outcome of HCQ treatment in their study of COVID-19 treatment. These findings (use of HCQ for COVID-19) are not sufficient to conclude the use of HCQ for COVID-19, thus important results and conclusion for the widespread adoption of HCQ against coronavirus disease-2019 are still awaiting. The individual uses of both these (HCQ and AZT) drugs are associated with QT Prolongation [33]. Hence QT prolongation is a major concern by synergetic use of HCQ + AZT in treatment of coronavirus disease-2019 [33]. Thus, there is an urgent need for the further reporting of case studies/clinical trials to get actual insights of clinical outcomes for possible use of HCQ against COVID-19 by considering an increasing widespread use of HCQ as a therapy as well as prophylaxis despite of its detrimental toxic effects. The major limitations of above mentioned studies is small sample size, random trials, short duration, limited follow-up, lack of control and no enrolment of children.

Table 2: Comparative account of literature review for the use of hydroxychloroquine in treatment of COVID-19

Study	Study design	Treatment and dosage	Control	Observation and general conclusion	Date publish/ Preprint	Ref
1	Open 1:1 controlled N = 30 MA = 50.5 and 46.7 TD: 5 days RN: NCT04261517	15 patients: HCQ (400 mg per day for 5 days) + conventional treatments MA = 50.5	15 patients: conventional treatment only MA = 46.7	No major distinction between both groups. One patient from HCQ group becomes severe ill during the treatment. On seventh day, throat swab for SARS-CoV-2 was found to be negative for 86.7% and 93.3% cases of HCQ group and control group respectively. Transient diarrhea and abnormal liver function appeared in 26.7% of the HCQ group and 20% of the control group.	Published 03-Mar-20	[5]
2	Open-label non-randomized clinical trial N = 36 MA =45 TD = 10 days RN: 2020-000890-25	14 patients: HCQ (600mg/day for 10 days), MA =51.2 On day six, 57.1% patients were virologically cured 6 patients: HCQ (600mg/day for 10 days) + AZT (500 mg on day one and then 250 mg per day upto fifth day), MA	16 patients: On day six, 12.5% patients were virologically cured in the control	Use of AZT along with HCQ showed more efficiency for elimination of virus load Limitation: Major limitation is small sample size, limited follow-up, exclusion and specific age group of patients. Reported death of one patient	Published 20-Mar-20	[6]

		=37.3 On day six, 100% patients were virologically cured		Any side effects are not reported.		
3	Observational study, non controlled N = 11 MA = 58.7 TD: 10 days	11 patients: HCQ (600 mg/day for 10 days)+ AZT (500 mg on day one and then 250 mg per day upto fifth day)	0	No indication of clinical benefit by HCQ + AZT treatment for severe COVID-19 patients. One patient died. The nasopharyngeal for SARS CoV-2 were found to be positive for SARS-CoV-2 in 80% patients on fifth day.	Published 30-Mar-20	[7]
4	Randomized clinical trial N = 62 MA =44.7 TD: 5 days RN:ChiCTR2000029559	31 patients: Hydroxychloroquin (400 mg/day for 5 days) + standard treatment MA =44.1	31 patients: standard treatment only MA =45.2	Reported clinical recovery, pneumonia absorption and cough reduction in HCQ treated group compared to control.	Pre-print 31-Mar-20	[8]
5	Uncontrolled non-comparative observational study N = 80 MA =53 TD: 10 days 2020 207 -000890-25	80 patients: HCQ (600 mg per day for ten days) + AZT (500 mg on first days and then 250mg upto fifth day)	0	On fifth day, 97.5% of patients showed negative virus cultures from respiratory sample. Reported death of one patient. Side effects of HCQ + AZT are determined by ECG study.	Published 11-Apr-20	[9]
6	Retrospective study N = 84 MA =63	84 patients: HCQ+AZT	NR	QT prolongation was observed in HCQ+AZT treated patients in between 3-4 days. 30% of patients showed QT prolongation by greater than 40ms, while 11% of patients showed QT increased to >500 ms which indicating the high risk arrhythmia. Reported death of four patients.	Pre-print 03-Apr-20	[10]
7	Multicenter, open-label, randomized controlled trial N = 150 N= 46.1 DT: 2-3 weeks RNChiCTR2000029868	75 patients: HCQ +SOC (1200 mg per day for 3 days and then 800 mg per day for 2-3 weeks) MA =48	75 patients: standard of care only MA =44.1	The medication of HCQ results in more alleviation of clinical symptoms than standard of care treatment. Adverse events were considerably increased in HCQ (30 %) recipients. No difference was observed in the main outcome of the HCQ group and control group.	Pre-print 14-Apr-20	[33]
8	Routine collected data N = 181 MA =60 TD: 7 days RN: 2020_060	84 patients: HCQ (600 mg/day for 2 days as only mentioned) + Standard care treatment	97 patients: (No HCQ) Standard care treatment	They mentioned that, clinical results did not support the HCQ use in COVID-19 patients. Further modification of ECG was observed in 9.5% patients in HCQ treated group. They reported death of 2.8% and 4.6% patients in HCQ treated and control patients respectively. 28.6 % and 24.2 % patients from HCQ and control group showed development of acute respiratory distress syndrome.	Pre-print 14-Apr-20	[34]
9	Retrospective analysis N = 368 MA =69	97 patients: (HCQ + standard supportive treatment), 113 patients: (HCQ + AZT + standard supportive treatment)	158 patients: standard supportive treatment only	They reported the rates of death as 27.8%, 22.1%, and 11.4%, in the HCQ, HCQ+AZT and control group respectively. Compared to control group, higher death ratio is observed in HCQ treated group. Rate of ventilation in HCQ, HCQ+AZT, and control groups were 13.3%, 6.9%, 14.1%, respectively. Suggested caution in HCQ and AZT use in treatment.	Pre-print 21-Apr-20	[35]

N: No. of population (patients), MA: median age in population (years); TD: treatment days,
RN: registration number

7. Prophylaxis use of HCQ

Interestingly, HCQ is allowed to use as prophylaxis in order to prevent possible infection of COVID-19 [27,36,37]. Despite of several detrimental adverse effects and lack of evidences the question arises regarding to prophylactic use and dosage of the HCQ. Most recently, dosing study of HCQ is optimized by Yao et al. [27], who reported EC₅₀ values (for HCQ) of 6.14 and 0.72 μM at 24 and 48 hours respectively [27]. On the basis of physiologically-based pharmacokinetic models, dosing of 400 mg BID (on day 1) and 200 mg BID (from day 2 to day 5) offers lung tissue trough concentration 21 and 83 times higher than EC₅₀ value at day 1 and day 10 respectively [27]. This study may indicated, effective use of HCQ as a prophylactic drug to prevent possible infection of COVID-19 on the basis of model and in-vitro analysis [27]. Moreover, cytokine storm (in COVID-19 infection) may be regulated by immunomodulatory action of HCQ [11-13]. However, looking to contradictory case results of use of HCQ against COVID-19 there is an urgent need for more confirmatory evidences [5-10,33-35].

HCQ may looks a promising life saving drug for in-vitro inhibition of coronavirus, however in practise it failed to reduce the actual effective corona virus load in mice [38]. Thus it is not confirmatory that whatever works in-vitro can be work well in-vivo, hence there is an urgent need to study clinical trials [38-42]. Further sudden increased use of HCQ as a prophylaxis despite of strong effective evidences may faces detrimental side effects and leads to create shortage of HCQ drug [36-38]. More, recently some hospitals of France as well as Sweden have stopped use of HCQ/CQ in clinical case study/practice considering the lack of therapeutic evidences,

detrimental side effects and poor clinical outcome [39,40]. It is mentioned by Ferari in media report Health-News that, use of HCQ +AZT sometimes may become more harmful than disease itself [39]. Moreover, recently, a death is reported by self-medicating use of CQ against COVID-19, hence, self-medication as a prophylaxis should be avoided which may be harmful or some-times may be lethal [41,42]. Prophylaxis use of HCQ should be done after medical (practitioner) advice only. Thus at present, more confirmatory results/study reports are still awaited for prophylaxis use as well as in-vivo clinical use of HCQ to treat COVID-19 [36-42].

8. Pharmacovigilliance approach for the use of HCQ:

Literature showing several adverse events associated with the use of HCQ. Although HCQ is less toxic and safer than CQ, still it possesses some detrimental adverse effects which are not negligible and cannot be ignored. In view of pharmacovigilance concern, unnecessary and excess use of the HCQ (without physician advise) may be dangerous which may have several side effects (Table 3, entries 1-6) such as cardiac complications (Table 3, entry 1) [43], ocular side effects (Table 3, entry 2) [44], myopathy, neuropsychiatric effects (Table 3, entry 3) [45], gastrointestinal discomfort (Table 3 entry 4) [46], erythema multiforme, exanthematic pustulosis (Table 3, entry 5) [47], hypoglycaemia (Table 3, entry 6) [48], Stevens-Johnson syndrome (Table 3, entry 5) [49], thrombocytopenia (Table 3, entry 6) [17] and other common side effects such as weight-loss, appetite-loss, dizziness, weakness, nausea, vomiting and allergic reactions etc. (Table 3, entry 6) [50]. Most of these side effects are associated with the long term use.

Table 3: Adverse effects of HCQ

Entry	Adverse effects	Ref.
1	Cardiac complications: QT prolongation, ventricular hypertrophy, ventricular arrhythmias, hypokinesia, cardiac arrest, pulmonary arterial hypertension, ventricular arrhythmias, valvular dysfunction, heart failure.	[43]
2	Ocular side effects: Retinopathy, blurred vision, blindness, corneal deposition	[44]
3	Neuro/muscular side effects: Signal conduction disorders, convulsion, myopathy, vertigo, psychosis, ataxia, nightmare, depression	[45]
4	Gastrointestinal side effects: Diarrhoea, vomiting, abdomen cramping, nausea, abnormal liver function	[46]
5	Cutaneous/allergic side effects: Erythema multiforme, exanthematic pustulosis, Stevens-Johnson syndrome, allergy, rashes.	[47,49]
6	General major side effects: Thrombocytopenia, hypoglycaemia, obnubilation, weight loss, appetite loss.	[48,50]

Conclusion:

In conclusion, HCQ shows antiviral effects against various viruses, further it's use showed immunomodulatory effect. HCQ may be safe to use and required in lower dosage than CQ to inhibit viral load, to regulate cytokine storm and time-span of viremia on the basis of in-vitro analysis. However, more clinical trials/ evidences are still awaited and required to prove in-vivo efficacy of HCQ against COVID-19. At present, clinical trials and case studies reported for the use of HCQ to combat COVID-19 showed the contradictory clinical outcome/results. QT prolongation is the major

concern when HCQ is combined with drugs which tackle lower respiratory tract infection (e.g. AZT). Adverse effects cannot be neglected in use of HCQ against COVID-19. Thus the use of HCQ (in COVID-19 treatment) is of current international interest, although a consensus has not yet been reached. Extreme care is required for the HCQ treatment in pregnant women, pediatric patient and patient with comorbidity in coronavirus disease COVID-19. Misuse of HCQ as a prophylaxis of COVID-19 (without physician's advise) should be avoided due to lack of strong evidences which may results into one or more detrimental side effects. Use of HCQ should be done after medical advice only. Considering

pharmacovigilance and public health concern, NO self medication of HCQ is advisable under any circumstances which may be risky and sometimes lethal. Thus the present review discussed the possible anti-viral mechanism of HCQ, prophylaxis strategy and effect of HCQ against SARS-CoV-2 virus in-vitro study. Further this review also summarizes and debates all available clinical trials/ case studies of HCQ use against COVID-19 (with clinical outcome). Finally possible detrimental adverse effects are also discussed considering the public health and pharmacovigilance concern.

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Note for readers: Readers must refer cited research article(s) for more detail information. Clinical treatment should NOT be given based on this review article.

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