

Systematic review and meta-analysis of efficacy and safety of hydroxychloroquine and chloroquine in the treatment of COVID-19

Niti Mittal^{1*}, Rakesh Mittal¹, M. C. Gupta¹, Jyoti Kaushal¹, Ankita Chugh²,
Daisy Khera³, Surjit Singh^{4*}

¹Department of Pharmacology, Postgraduate Institute of Medical Sciences, Rohtak, Haryana, ²Departments of Dentistry, ³Pediatrics and ⁴Pharmacology, All India Institute of Medical Sciences, Jodhpur, Rajasthan, India
*- Dr N Mittal and Dr S Singh contributed equally to this article

ABSTRACT

Repurposed drugs like hydroxychloroquine (HCQ) and chloroquine (CQ) are being tested for potential therapeutic role in COVID-19. We aimed to evaluate efficacy and safety of HCQ and CQ in COVID-19. Using PubMed, EMBASE, medRxiv, Google Scholar, clinicaltrials.gov, electronic search was carried out to identify relevant articles till June 2020 with re-evaluation in last week of November 2020. Observational and interventional clinical studies comparing efficacy of CQ or HCQ to standard management or other drug/s for SARS-CoV-2 infection patients were included. Cochrane review manager version 5.3 was used for synthesis of meta-analysis results. For randomized controlled trials, risk of bias was assessed using Cochrane Collaboration risk of bias assessment tool, version 2.0 (ROB-2). ROBINS-I was used for quality assessment of observational studies. Overall evidence quality generated by review was graded as per GRADE Recommendation. A total of 903 studies were screened. Nineteen studies were included in synthesis of meta-analysis with total of 4,693, 1,626, and 6,491 patients in HCQ/CQ, HCQ/CQ+AZ and control groups, respectively. HCQ/CQ treatment was associated with significantly increased rates of virological cure (OR = 2.08, 95%CI = 1.36-3.17; $P = 0.0007$) and radiological cure (OR = 3.89, 95%CI = 1.35 - 11.23; $P = 0.01$) compared to control. HCQ/CQ had no difference in unadjusted mortality rate (unadjusted OR = 0.98 95% CI = 0.70-1.37, $P = 0.89$, random effect model) and adjusted hazard ratio for mortality (adjusted HR = 1.05, 95%CI = 0.86-1.29; $P = 0.64$). However, a significant increase in odds of disease progression (OR = 1.77, 95%CI = 1.46-2.13; $P < 0.00001$) and QT prolongation (OR = 11.15, 95%CI = 3.95-31.44; $P < 0.00001$) was noted. The results with HCQ/CQ and azithromycin combination were similar to HCQ/CQ mono-therapy. In the light of contemporary evidence on effectiveness of HCQ/CQ, judicious and monitored use of HCQ/CQ for treatment of COVID-19 patients is recommended in low to middle income countries with emphasis on no mortality benefit.

Registration number of Systematic review. Register in PROSPERO database: CRD42020187710

Keywords: Azithromycin, chloroquine, COVID-19, hydroxychloroquine, SARS-CoV-2

Introduction

Infection due to a newly detected β -coronavirus was identified as responsible for an outbreak of pneumonia cases in Wuhan,

Address for correspondence: Dr. Surjit Singh,
Department of Pharmacology, AIIMS, Jodhpur, Rajasthan, India.
E-mail: sehmy_ss@yahoo.com

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China during December 2019.^[1] Due to its similarity to SARS-CoV (responsible for major coronavirus outbreak in 2003) on genome sequencing, this novel coronavirus was labelled as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and the resulting illness as coronavirus disease-2019 (COVID-19).^[2]

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Multiple clinical trials are being conducted internationally to come forward with effective treatment options for COVID-19. Besides newer investigational strategies being explored, current emphasis is also on few repurposed drugs like lopinavir/ritonavir, oseltamivir, ribavirin, interferons, chloroquine, hydroxychloroquine, etc.^[3] In particular, hydroxychloroquine (HCQ) and chloroquine (CQ) have received much media coverage after being labeled as potential “game changers” by American President for COVID-19 treatment.

CQ and HCQ have a long lasting history as effective antimalarial and immuno-modulatory agents. HCQ, a derivative of CQ with an extra hydroxyl group, has a superior safety profile^[4] as compared to chloroquine. *In vitro* studies have reported inhibitory potential of CQ and HCQ against SARS-CoV^[5] and SARS-CoV-2.^[6] CQ/HCQ have been reported to block multiple steps in viral life cycle, for example, viral binding and entry into cell, release of viral genome, viral replication, virion assembly and budding.^[7] Hence, HCQ/CQ furnish a promising avenue in treatment of COVID-19

Despite the fact that HCQ/CQ have shown remarkable results against SARS-CoV-2 in *in vitro* studies, confirmatory findings from clinical trials are mandatory to bring about an evidence for utilizing them as weapons to combat COVID-19 infection. The current review was conducted with an objective to evaluate the therapeutic potential of CQ/HCQ given alone and in combination with azithromycin for treatment of confirmed COVID-19 infection caused by SARS-CoV-2, in comparison to standard management or other drugs.

Material and Methods

Protocol registration

The current systematic review was conducted on basis of PRISMA “(Preferred Reporting Items for Systematic Reviews and Meta-Analyses)” statement and “Cochrane guidelines for Systematic Reviews of Interventions”. Prospective registration of review was done in the database of the “International Prospective Register of Systematic Reviews (PROSPERO)” (protocol number CRD42020187710).

Study eligibility criteria

Observational (prospective/retrospective, case-control/cohort) and interventional clinical studies [randomized clinical trials (RCT)] assessing the efficacy and safety of HCQ/CQ in comparison to standard management or drug/s other than HCQ/CQ for treatment of SARS-CoV-2 infected patients were considered eligible for inclusion. Case reports, case series, expert opinions, literature review articles, *in vitro* and non-clinical studies were excluded.

Search strategy and study selection

Electronic search was done using PubMed, EMBASE, Google Scholar, preprint database like medRxiv and registry for clinical

trial, that is, clinicaltrials.gov to identify pertinent articles till 15th June, 2020. Reevaluation of literature was done for any completed RCTs in last week of November, 2020. We manually conducted bibliographic search to identify other relevant studies. No restriction with respect to language and publication status was followed. Search strategy was developed consisting of these key terms and other associated MeSH (medical subject headings) terms: “hydroxychloroquine,” “chloroquine,” “COVID,” “novel coronavirus,” “SARS-CoV-2,” “COVID-19.”

Two independent researchers assessed the titles and abstracts collected for their potential inclusion and removed duplicates. For the eligible studies, quality assessment of full text articles was carried out by two authors independently. Any dissent between authors was sorted out by agreement or discussion with third review author.

Study data extraction

Two review authors extracted data on a structured form tested a priori consisting of items regarding study in general, institution or country of conduct, design, interventions, participants, efficacy, and safety outcomes.

Study outcomes

The primary efficacy objectives were virological cure (defined as negative RT-PCR test for SARS-CoV-2 RNA) and mortality (number of deaths per group). The secondary outcomes included clinical improvement (defined as relief or alleviation of respiratory symptoms, fever, improvement in SpO₂), radiological cure (pulmonary findings on CT scan), discharge from hospital (defined as number of patients discharged in each group), disease progression (defined as ICU admission, need for intubation, increased severity of illness or radiological progression on CT scan). Safety outcomes reported were general and cardiovascular (cardiac arrest or arrhythmias or QT prolongation) adverse events.

Quality assessment of studies

Two researchers independently appraised methodological quality of studies according to “Cochrane Collaboration risk of bias assessment tool, version 2.0 (ROB-2)” for RCTs^[8] and ROBINS-I (“The Risk Of Bias In Non-randomized studies of Interventions”)^[9] guidelines for observational studies. The plots for risk of bias were synthesized using Robvis (visualization tool).^[10]

Publication bias was assessed using funnel plot. Egger’s regression test was also performed.

Data synthesis assessment and outcome measures

For summarizing dichotomous data, odd ratios (OR) and adjusted hazard ratios (AHR) with 95% confidence intervals (CI) were used as applicable. All analyses were conducted using Review Manager Version 5.3. software. Heterogeneity was assessed using I² statistic with I² of 25, 50, and 75% representing low, medium, or large heterogeneity.^[11] For significant (I²>50%) heterogeneity,

sensitivity analysis was performed after exclusion of studies. The results were presented using fixed effect model. For significant heterogeneity, analysis, and interpretation with random effect was also assessed if sensitivity analysis with exclusion of studies was not possible.^[11,12]

Evidence quality as per GRADE Pro

Quality of evidence of review was evaluated using GRADE pro GDT (guideline development tool) software,^[13,14] using parameters like study design, ROB, directness of outcomes, heterogeneity, precision within results, bias due to publication, estimate effect, dose relationship with response and confounders. Optimal information size (OIS) was derived as 245 subjects in either of the groups. Overall GRADE thus obtained can be high quality, moderate, low or very low quality evidence.

Results

Study selection

Study inclusion process has been represented using PRISMA flow chart [Figure 1]. Of the total 903 records screened, 18 studies were assessed in qualitative (systematic) and 19 (7 randomized

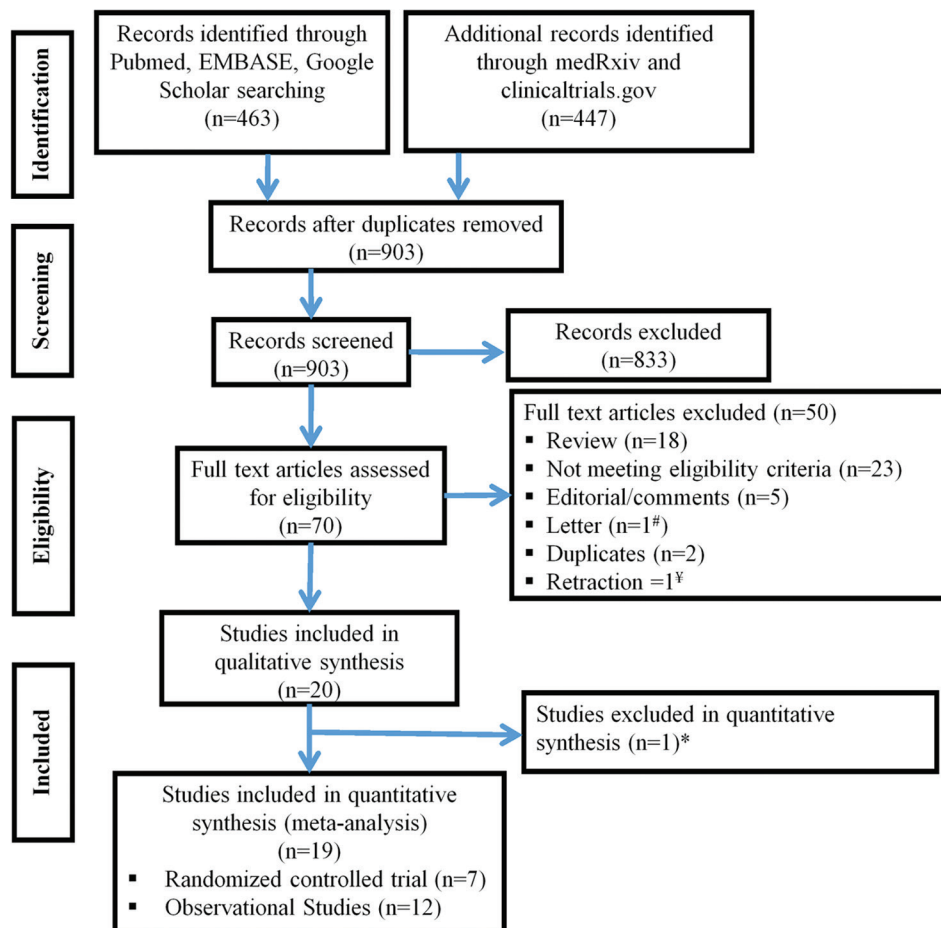
controlled trials^[15-21] and 12 observational studies^[22-33]) in quantitative analysis. Study by Mehra *et al.* was excluded because of retraction of article by the authors.^[34] Due to non-availability of data on study design, patients, and outcomes, Gao *et al.*^[35] was excluded. Absence of efficacy data with HCQ/CQ (alone or combined with azithromycin) exclusive of other antivirals, precluded the inclusion of Shabrawishi *et al.*^[36] study in quantitative analysis. One study^[17] published in Chinese language was translated into English using Google translator web service prior to review conduct.

Study characteristics

Table 1 depicts the characteristics of various RCTs and observational studies (OS) included in this systematic review and outcomes data reported therein.

Methodological Quality - Risk of bias (ROB)

Among RCTs, overall ROB was recorded as high for Barbosa *et al.* (quasi-randomized design),^[15] some concerns for Chen J *et al.*^[17] and Huang *et al.* RCT^[19] (lack of details on allocation concealment in both), and low for Chen Z *et al.*,^[18] Tang *et al.*,^[16] WHO solidarity trial^[21] and recovery



¥ - Mehra et al, # - Gao et al, * - Shabrawishi et al

Figure 1: PRISMA flow chart depicting study selection process

Table 1: Characteristics of clinical studies evaluating HCQ/CQ for treatment of COVID-19

Study ID (Study design)	Institution/Country of study conduct	Study Interventions (n)/Regimen	Study control (n)/Regimen	Study population characteristics	Study outcomes
Barbosa et al. 2020 ^[5] (Quasi-randomised, open label, parallel group trial)	University School of Medicine, Michigan, USA	HCQ (32) HCQ: Loading dose (400 mg orally BD x 1-2 days) followed by 200-400 mg daily dose x 3-4 days	Standard supportive care (31)	Males: 37 (58.7%); Age: 62.7±15.1 years	Change in respiratory support level at 5 days: HCQ: 0.63±0.79, Supportive care: 0.16±0.64; P=0.013; Change in Absolute Lymphocyte Count (K/ μ l): HCQ: -0.16±0.52, Supportive care: -0.61±0.38; P=0.413; Change in neutrophil to lymphocyte ratio: HCQ: +9.55±21.5, Supportive care: +1.58±6.26; P=0.051; Mortality: HCQ: 4/31 (12.9%); Supportive care: 1/32 (3.13%); P=0.196; Torsades de pointes: no reports during study timeframe.
Tang et al. 2020 ^[6] (Multi-center, parallel, open-label, randomized, trial)	China	HCQ (75) HCQ: loading dose of 1200 mg daily for 3 days followed by 800 mg daily dose for 2 or 3 weeks (for mild-moderate or severe disease)	Standard of care (SOC) (75)	Males: 82 (54.7%); Mean age: 46.1±14.7 years (Mild to moderate disease, n=148; severe disease, n=2)	28-day negative conversion rate of SARS-CoV-2: 85.4% (73.8-93.8%) in HCQ vs 81.3% (71.2-89.6%) in SOC only arm; P=0.341 (similar negative conversion rates for the two groups at days 4, 7, 10, 14, and 21). Symptom relief at 28 days (fever, respiratory symptoms, SpO ₂): 59.9% (45-75.3%) with HCQ vs 66.6% (39.5-90.9%) with SOC alone; Adverse events: 30% in HCQ vs 8.8% in SOC arm; diarrhea most common, 2 serious adverse events in HCQ arm (disease progression, upper respiratory tract infection)
Chen J et al. 2020 ^[7] (Randomised controlled trial)	Fudan University, Shanghai, China	HCQ (15) HCQ 400 mg daily for 5 days	Standard treatment (15)	Males: 21 (70%); Mean age: 50.5±3.8 yrs in HCQ, 46.7±3.6 yrs in control group	Virologic clearance at day 7: 13 (86.7%) cases in the HCQ group and 14 (93.3%) cases in the control group (P>0.05); Median time for body temperature normalization: HCQ: 1 (0-2) days, control: 1 (0-3) days; Radiological progression on CT scan: 5 cases (33.3%) of the HCQ group and 7 cases (46.7%) of the control group; Adverse events: 4 (26.7%) in HCQ group vs 3 (20%) in control group (transient diarrhea and abnormal liver function); P>0.05
Chen Z et al. 2020 ^[8] (Randomised, double blind, controlled trial)	Renmin Hospital of Wuhan University, Wuhan, China	HCQ (31) HCQ : 400 mg daily for 5 days	Standard care (31)	Males: 29/62 (46.8%) Age (mean±SD): 44.7 (15.3) years	Time to clinical recovery (TTCR): Body temperature recovery time [2.2 (0.4) days vs 3.2 (1.3) days] in HCQ vs control group (P=0.0008); cough remission time [2 (0.2) vs 3.1 (1.5) days] in HCQ vs control group (P=0.0016). Progression to severe disease: 4 patients in control vs 0 in HCQ. Radiological improvement in pneumonia: 25 (80.6%) vs 17 (54.8%) in HCQ and control groups respectively. Adverse events: HCQ group: 2 patients (mild rash, headache)
Huang M et al. 2020 RCT ^[9] (Randomised controlled trial)	Sun Yat-sen University, Zhuhai, China	CQ (10) CQ: 500 mg orally BD for 10 days	Lopinavir/ritonavir (12); 400/100 mg orally BD x 10 days	Males: 13/22 (59%) Age: 44.0 (36.5-57.5) years	Virologic cure: 10 (100%) in CQ vs 11 (91.67%) in Lop/ritgp. Hospital discharge at Day 14: 10 (100%) vs 6 (50%) in CQ and Lop/ritgp; Clinical recovery at Day 10: 8 (80%) vs 7 (58.33%) in CQ and Lop/rit gp; CT scan improvement at day 14: 10 (100%) vs 9 (75%) in CQ and Lop/rit group; Adverse events: 5 patients with 9 adverse events in CQ group (diarrhea and vomiting most common), no serious adverse events reported
Gautret et al. 2020 OS ^[2] (Non-randomised, open label, comparative trial)	IHU-Méditerranée Infection, Marseille, France	HCQ (14), HCQ+AZ (6), HCQ: 600 mg daily for 10 days; AZ: 500 mg on day 1, 250 mg daily for next 4 days	Controls (not receiving HCQ from other centers and those not giving consent) (16)	Males=15 (41.7%); Mean age: 45.1±22.0 years	Virologic clearance at day 6 post inclusion: HCQ: 57.1% (8/14), HCQ + AZ: 100% (6/6), Control: 12.5% (2/16); P<0.001; Mortality: 1/20 (5%). 6 HCQ patients lost to follow up (3 transferred to ICU, 1 died, 1 left the hospital and 1 stopped treatment due to nausea).
Cipriani et al. 2020 ^[23] (Observational case-control study)	University of Padua Medical school, Italy	HCQ + AZ (22) HCQ (200 mg, twice daily) and AZ (500 mg, once daily)	Healthy matched controls	Males: 18 (82%); Age [median (IQR)]: 64 (56-70)	QTc interval (ms): 450 (416-476) after therapy vs 426 (403-447) before therapy; P=0.02; QTc >480 ms; n (%): 4 (18) after vs 0 before therapy; P=0.04

Contd...

Table 1: Contd...

Study ID (Study design)	Institution/Country of study conduct	Study Interventions (n)/Regimen	Study control (n)/Regimen	Study population characteristics	Study outcomes
Rosenberg et al. 2020 ^[24] (Retrospective multicenter cohort)	25 hospitals in New York metropolitan region	HCQ (271), AZ (211), HCQ+AZ (735)	Supportive (221)	Males: 858 (59.7%) Age (median): 63 years	In-hospital mortality: (unadjusted analyses) HCQ + AZ (n=189, 25.7% [95% CI, 22.3%-28.9%]), HCQ (n=84, 19.9% (15.2-24.7%)), AZ (n=21, 10.0% (5.9-14.0%)), and neither-drug (n=28, 12.7% (8.3-17.1%)) (P<0.001); (adjusted analysis) no significant difference in mortality between groups. Cardiac arrest: more likely in HCQ + AZ group (adjusted OR, 2.13 (1.12-4.05), but not HCQ (1.91, 0.96 to 3.81) or AZ (0.64, 0.27 to 1.56)). Abnormal ECG findings: no significant differences in the relative likelihood between different groups.
Gelenis et al. 2020 ^[23] (Observational study)	New York-Presbyterian Hospital (NYP)-Northern Manhattan.	HCQ (811) (600 mg twice on day 1, then 400 mg daily for a median of 5 days)	No HCQ (505)	Males: 778 (56.5%)	Composite end point of intubation or death: HCQ: 262 (32.3%), No HCQ: 84 (14.9%); hazard ratio: 2.37 (1.84-3.02); no significant association between HCQ use and intubation or death (hazard ratio, 1.04, 0.82-1.32)
Magnoli et al. 2020 ^[26] (Retrospective cohort study)	US Veterans Health Administration medical centers	HCQ (198), HCQ+AZ (214)	Standard care only (395)	Males: 772 (95.6%); Age [median (IQR)]: 71 (62-76.8) in HCQ, 68 (59-74) in HCQ+AZ, 70 (59-77) yrs in control group.	Mortality rate (%): HCQ (19.2), HCQ+AZ (22.9), No HCQ (9.4); P<0.001. Rate of ventilation (%): HCQ (19), HCQ+AZ (20.5), No HCQ (19.9); P=0.94. Risk of ventilation: HCQ (aHR, 1.19, 95% CI, 0.78-1.82; P=0.42); HCQ+AZ (1.09; 0.72-1.66; P=0.69), compared with no HCQ group. Risk of death from any cause: HCQ (aHR, 1.83; 1.16-2.89; P=0.009); HCQ+AZ: (1.31; 0.80-2.15; P=0.28) compared with no HCQ group
Mahevas et al. 2020 ^[27] (Retrospective cohort study)	4 tertiary hospitals, France	HCQ (n=84) 600 mg daily	No HCQ (n=97)	Males: 71.1%; Age [median (IQR)]: 60 (52-68) years (Patients with COVID-19 pneumonia requiring oxygen but not intensive care)	Survival rate without transfer to ICU at day 21: 76% in HCQ group and 75% in control group; weighted hazard ratio (95% CI): 0.9 (0.4-2.1). Overall survival at day 21: 89% in HCQ and 91% in control group (1.2, 0.4 to 3.3); Survival without ARDS at day 21: 69% in HCQ vs 74% in control group (1.3, 0.7 to 2.6); Weaning from oxygen at day 21: 82% vs 76% in HCQ vs control group (1.1, 0.9 to 1.3); ECG modifications requiring treatment discontinuations: 8 (10%) in HCQ group
Yu et al. 2020 ^[28] (Retrospective cohort)	Wuhan, China	HCQ (48) Low dose - 200 mg BD x 10 days	Standard treatment (502)	Critically ill patients; Males: 62.5% Age: 68 (59-77) years	Mortality: 18.8% (9/48) in HCQ group, 47.4% (238/502) in NHCQ group (P<0.001); Duration of hospitalization before death: 15 (10-21) days and 8 (4-14) days for the HCQ and NHCQ groups, respectively (P<0.05). Change in inflammatory cytokine IL-6 levels: Significant reduction from 22.2 (8.3-118.9) pg mL ⁻¹ at the beginning of treatment to 5.2 (3.0-23.4) pg mL ⁻¹ (P<0.05) at the end of treatment in HCQ group, no change in NHCQ group.
Hraïch et al. 2020 ^[29] (Retrospective case-control study)	France	HCQ+AZ (17)	Lopinavir/ritonavir (13), Standard care (15)	Patients with SARS-CoV-2 related moderate to severe ARDS Males: 35 (77.8%); Age: 60±17, 62±13 and 60±16 yrs in HCQ+AZ, Lop/rit and control groups, respectively	Negative SARS-CoV-PCR at day 6 of treatment: 3 (18%) in HCQ + AZ, 5 (38%) in Lop/rit and 2/10 patients assessed (20% in control group; P=0.39; Negative SARS-CoV-PCR at day 6 from ARDS: 2 (12%) in HCQ + AZ, 5 (38%) in Lop/rit and 2 (13%) in control group; P=0.14; Mortality: 2/45 (4.4%), both deaths in HCQ + AZ group
Huang M et al. 2020 OS# ^[30] (multicenter prospective observational study)	China	CQ (197) CQ phosphate 500 mg OD/BD	Non-CQ (176)	COVID-19 patients (excluding critically ill); Males: 373 (53.1%); Age: 43.8±13.1 in CQ; 45.6±13.5 yrs in non-CQ group	Time from treatment initiation to undetectable viral RNA, median (IQR) days: 3 (3-5) in CQ vs 9 (6-12) in non-CQ group; P<0.0001; Duration of hospitalization, median (IQR) days: 19 (16-23) in CQ vs 20 (15.8-24) in non-CQ group; P=0.25; Adverse events: 53 patients (26.9%) in CQ group and 57 (32.4%) in non-CQ group; no serious adverse event

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Table 1: Contd...					
Study ID (Study design)	Institution/Country of study conduct	Study Interventions (n)/Regimen	Study control (n)/Regimen	Study outcomes	
Mallat <i>et al.</i> 2020 ^[31] (Retrospective observational study)	Cleveland Clinic Abu Dhabi	HCQ (23) 400 mg BD x 1 day followed by 400 mg daily x 10 days	Non-HCQ (11)	COVID-19 patients with mild to moderate disease; Males: 25 (73.5%); Age: 37 (31-48) yrs Males: 991 (54.4%) Mean age: 62.17±16.81 and 62.55±17.62 yrs in HCQ and control groups, respectively.	Time to SARS-CoV-2 negativity test: 17 [13-21] vs. 10 [4-13] days in HCQ vs non-HCQ groups; P=0.023; Duration of hospitalization: 17 (6-20) vs 9 (6-12.7) days in HCQ vs non-HCQ groups; P=0.068 HCQ vs control Mortality 30 day: 11.43% in HCQ vs 11.98% in control group. Mechanical ventilation: 5.05% in HCQ vs 6.26% in control group. HCQ+AZ versus control. Mortality 30 day: 12.27% in HCQ + AZ vs 10.27% in control group. Mechanical ventilation: 5.71% in HCQ + AZ vs 5.85% in control group.
Membrillo <i>et al.</i> ^[33] Observational cohort study	Central Defense Hospital "Gómez Ulla", Madrid, Spain	HCQ (123)	No HCQ (43)	Males: 103 (62%) Mean age: 61.6±16.2 and 68.7±18.8 yrs in HCQ and control groups, respectively.	Mean cumulative survival: Mild group- 14.4 days (95% CI: 13.7-15.2 days) in HCQ, 8.2 days (95% CI: 6.5-9.9 days) in control arm; P=0.032 Moderate group- 10.9 days (9.3-12.5) in HCQ, 7.7 days (4.4-10.9) in control arm; P=0.205 Severe group- 6 days (3.3-8.5) in HCQ, 4 days (1.7-6.1); P=0.297 Virological cure, n (%): 33 (73.3) in intervention group vs 33 (68.8) in control group, P=0.655
Shabrawishi <i>et al.</i> ^[36] Retrospective cohort study	Tertiary hospital in Mecca, Saudi Arabia	HCQ (15), HCQ + AZ (25), HCQ + antivirals (5)	Supportive care (48)	Males: 49 (52.7%) Mean age: 43.9±15.9 yrs	Mortality rate=rate ratio, 1.09; 95% CI - 0.97 to 1.23; P=0.15 Invasive mechanical ventilation or death=risk ratio, 1.14; 95% CI, 1.03-1.27
RECOVERY Trial RCT (20)	Multicentric, World	HCQ (1561)	Standard of care (3155)	Mean age: HCQ=65.2±15.2 Control=65.4±15.4	104 of 947 - HCQ; 84 of 906 - Control Mortality rate=rate ratio, 1.19; 95% CI - 0.89 to 1.59; P=0.23
WHO Solidarity Trial RCT ^[21]	Multicentric, WHO	HCQ (947)	Standard of care (906)	More number of patients having lesions in both lungs and on supplemental oxygen in HCQ group.	

*RCT (Randomised controlled trial), #OS (Observational study), ARBs: angiotensin receptor blockers, NEWS: National Early Warning Score

trial.^[20] [Figure 2a]. For observational studies (OS), ROB was low for Singh *et al.*^[32] and Cipriani *et al.*,^[23] moderate for five studies^[24-28] and serious for five studies^[22,29-31,33] (2 had missing data,^[22,30] and one had moderate concerns with selection of participants^[31] [Figure 2b]. Hence, overall ROB for OS was judged as moderate to high.

Efficacy outcomes

Virological cure

In pooled analysis, we observed a statistically significant increase in virological cure rate with HCQ/CQ compared

to control [OR (95% CI) = 2.08 (1.36–3.17), *P* = 0.0007; *I*² = 80%] [Figure 3]. Data was derived from three RCTs and three OS including 340 and 305 patients in HCQ/CQ and control groups, respectively. In sub-group analysis, significantly improved virological cure rates with HCQ/CQ vs control was observed only with OS [OR = 4.03 (2.22–7.32), *P* < 0.00001; *I*² = 83%] [Figure 3] and not RCTs [OR = 0.83 (0.43–1.62), *P* = 0.59; *I*² = 0%] [Figure 3]. Sensitivity analysis on excluding the study by Mallat *et al.* (moderate selection bias) resulted in *I*² = 0% among OS without any change in overall virological cure [OR = 7.10 (3.44–14.67), *P* < 0.00001; *I*² = 0%].



Figure 2: a: RoB-2: Risk of bias in randomized clinical trials evaluating HCQ/CQ in the treatment of COVID-19. b: ROBINS-I: Risk of bias in observational studies evaluating HCQ/CQ in the treatment of COVID-19

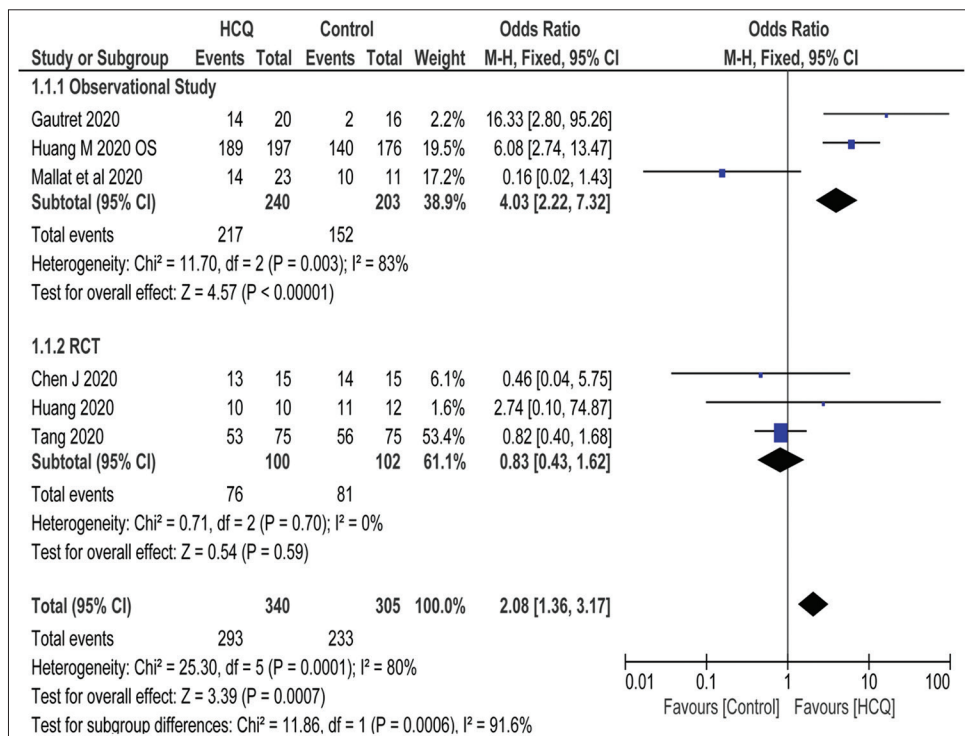


Figure 3: Virological cure (HCQ/CQ vs control treatment)

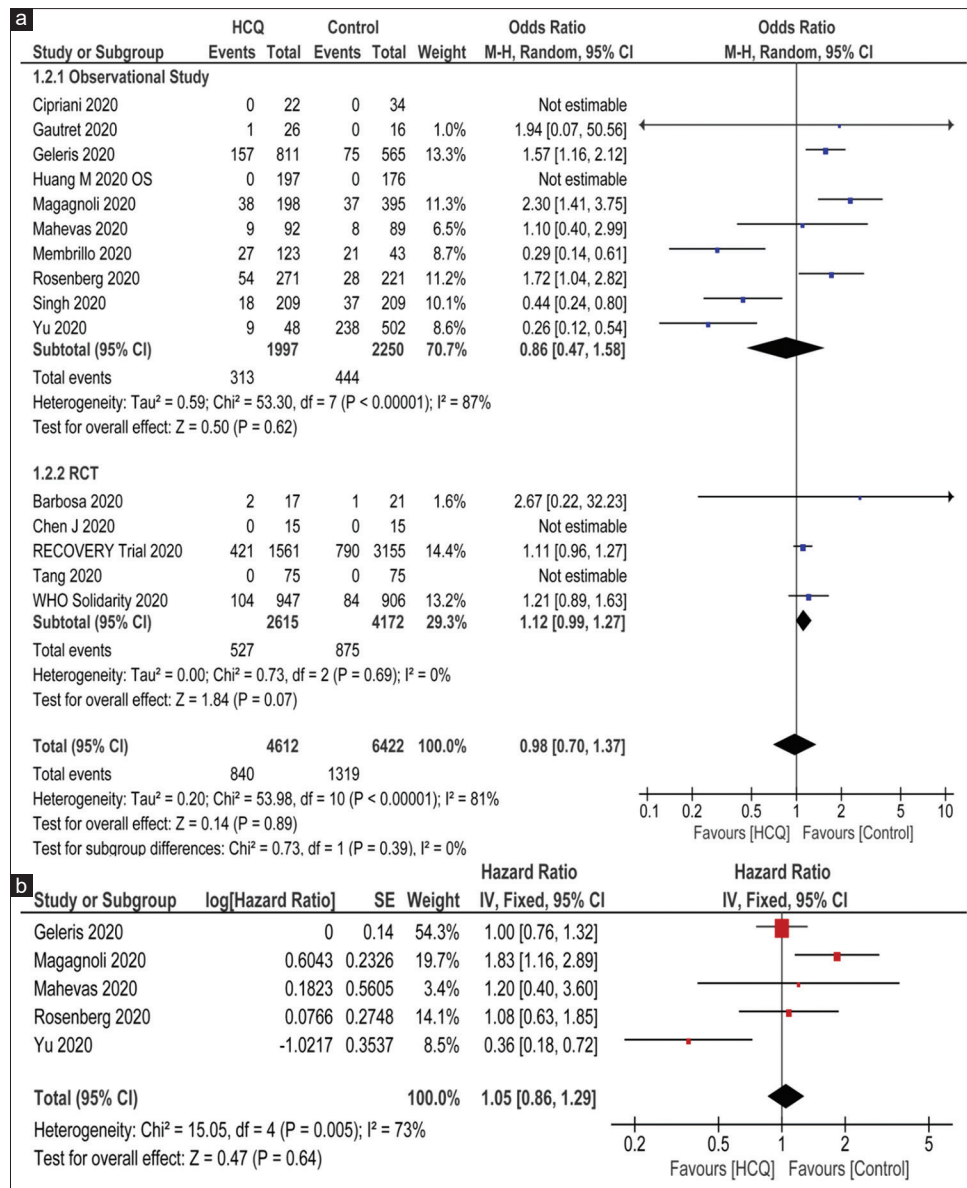


Figure 4: ab: Mortality rate (unadjusted-4a)(adjusted-4b) (HCQ/CQ vs control treatment)

Mortality [Mortality rate (MR) or Hazard ratio (HR)]

For unadjusted MR, pooled results demonstrated increased risk of mortality with HCQ/CQ compared to control [OR (95% CI) = 1.12 (1.01–1.24), P = 0.03; I² = 81%, fixed effect model]; data obtained from five RCTs and 10 OS comprising of 4,612 and 6,422 patients in HCQ/CQ and control groups, respectively. Sensitivity analysis with random effect model presented no significant difference in overall mortality [OR = 0.98 (0.70–1.37), P = 0.89; I² = 81%] [Figure 4a]. Pooled analysis of adjusted MR showed no increase in adjusted HR with HCQ/CQ versus control [Figure 4b] [HR (95% CI) = 1.05 (0.86–1.29), P = 0.64; I² = 73%; 5 OS]. Sensitivity analysis on excluding the study by Yu *et al.*^[28] showed similar results [HR = 1.16 (0.94–1.43), P = 0.17; I² = 40%; 4 OS].

For HCQ/CQ + AZ combination, there was a statistically significant rise in unadjusted MR compared to control, [unadjusted

OR = 1.84 (1.47–2.31), P < 0.00001; I² = 73%; 4 OS] [Figure 1Sa]. Sensitivity analysis excluding the study by Singh *et al.*^[28] showed similar results [HR = 2.59 (1.89–3.56), P < 0.00001; I² = 0%; 3 OS].

However, the result was insignificant for adjusted mortality rate between groups [Adjusted HR = 1.33 (0.91–1.93), P = 0.14; I² = 0%; 2 OS] [Figure 1Sb].

Disease progression

Pooled analysis of nine studies revealed a statistically significant increase in rate of disease progression [Figure 5] with HCQ/CQ treatment versus standard of care [OR = 1.77 (1.46–2.13), P < 0.00001; I² = 78%]; results included from three RCTs and six OS comprising of 1,646 patients in HCQ/CQ and 1,627 in control group.

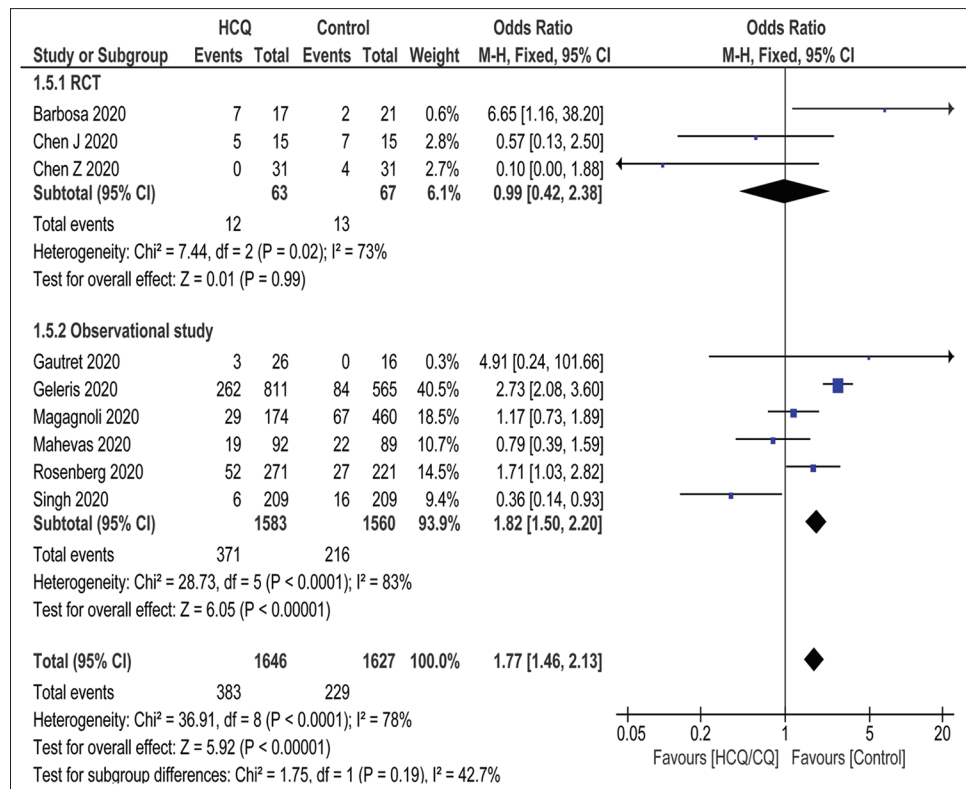


Figure 5: Number of patients showing evidence of disease progression (HCQ/CQ vs control treatment)

HCQ/CQ + AZ resulted in statistically significant increased odds of disease progression compared to control [OR = 1.74 (1.36–2.22), $P < 0.0001$; $I^2 = 81\%$; 4 OS] [Figure 2S].

Radiological cure

In pooled analysis, HCQ/CQ resulted in significant increase in odds of radiological improvement compared to control [OR = 3.89 (1.35–11.23), $P = 0.01$; $I^2 = 0\%$]; results obtained from two RCTs with 41 and 43 individuals in HCQ and control groups, respectively [Figure 3S].

Clinical improvement

Number of subjects achieving clinical improvement were similar in HCQ/CQ and control groups [OR = 0.89 (0.45–1.77), $P = 0.74$; $I^2 = 40\%$]; results extracted from 2 RCTs with a total of 76 and 65 individuals in control and HCQ/CQ groups, respectively [Figure 4S].

Hospital discharge

In pooled analysis, standard of care resulted in 36% increase in odds of discharge from hospital in comparison to HCQ/CQ treatment [OR = 0.64 (0.53–0.78), $P < 0.00001$; $I^2 = 86\%$]. Results were derived from one RCT and four OS with 1,234 and 1,104 individuals in HCQ and control arms, respectively [Figure 5S].

Safety outcomes

Cardiovascular adverse events

Similar to mortality rate, there was a significant rise in odds of QT prolongation [Figure 6S] in subjects administered HCQ/CQ

versus control [OR = 11.15 (3.95–31.44), $P < 0.00001$; $I^2 = 0\%$]; results obtained from 3 OS comprising of 347 and 278 patients in HCQ group and controls, respectively. In Rosenberg *et al.*^[24] HCQ + AZ resulted in significant increase in number of individuals with QT prolongation [OR = 7.32 (2.28–23.49), $P = 0.0008$]

Rosenberg *et al.* showed no difference in number of individuals having cardiac arrest or arrhythmias in HCQ/CQ versus controls arms [unadjusted OR = 1.67 (0.66–4.20), $P = 0.28$; 1OS]; adjusted OR = 1.91 (0.96–3.80), $P = 0.07$; 1OS]. However, HCQ/CQ + AZ resulted in 1.52 times (1.13 times in adjusted analysis) increase in number of events versus control [unadjusted OR = 2.52 (1.44–4.42), $P = 0.001$; 1OS; adjusted OR = 2.13 (1.12–4.05), $P = 0.02$].

Other adverse events

There was no difference in adverse events in two treatment groups (OR = 1.26, 0.93–1.70; $P = 0.14$; $I^2 = 52\%$) [Figure 7S]. In sub-group analysis, significant increase in adverse events were reported in HCQ/CQ group as compared to control in RCTs only and not in OS.

Publication bias

Overall publication bias was regarded as low for current review. The funnel plot of 15 studies included for mortality rate estimation appears asymmetrical [Figure 8S], however, Egger's regression test indicated low publication bias, with t value = -0.5415 and P value = 0.5974. Egger's regression

test for virological cure ($t = -0.2039$, P value = 0.8484), disease progression ($t = -1.5724$, P value = 0.1599), discharge from hospital ($t = 2.2800$, P value = 0.1069), prolonged QT interval ($t = 3.3295$, P value = 0.1858) indicated low bias for publication. Therefore, overall bias due to publication has been considered as low.

GRADE analysis using GRADE Pro GDT

The GRADE Pro GDT recommendation for primary objective, that is, unadjusted MR was “Very Low” evidence quality as there were serious issues with ROB of included studies, inconsistency, and imprecision. The ROB for adjusted MR was considered as low as adjustment for confounding factors was done during analysis. Sensitivity analysis with exclusion of Yu *et al.* had resulted in heterogeneity of 31%, hence no serious inconsistency. Therefore, GRADE was analyzed as “Moderate” quality evidence for adjusted MR. The GRADE recommendation for virological cure and radiological cure was “Moderate” evidence quality, due to serious concerns in ROB and imprecision, respectively. The GRADE analysis for progression of disease and hospital discharge were graded as “Low evidence” quality because of serious concerns in ROB and heterogeneity. The prolonged QT interval was graded as having “High” quality evidence because of large effect size which confers strong association of outcomes with intervention. The results of quality of evidence as per guiding principles of GRADE Pro are shown in Table 2.

Discussion

Notwithstanding the present predicament on whether the uncertain efficacy from these drugs is worth the clear risks they pose to infected patients, HCQ/CQ are being recommended worldwide alone or in combination with azithromycin in a compassionate manner as an unproven COVID-19 treatment cocktail. National Health Commission of the People’s Republic of China recommended CQ phosphate for COVID-19 treatment based upon a preliminary report with unpublished results^[35] which was later included in other international guidelines as well,^[37,38] However, we as a medical community need to be wise enough to pause and appraise the evidence before using them indiscriminately. Hence, this systematic review was conducted to evaluate overall efficacy and safety of CQ/HCQ in confirmed COVID-19 patients in comparison to standard management or other drugs.

In our review, we found statistically significant increase in virological cure in HCQ/CQ compared to control group; the results were mainly attributed to OS because of insignificant results with RCTs on subgroup analysis. However, overall moderate quality evidence as per GRADE suggests possibility of change in effect estimate with the inclusion of more well conducted studies. An explanation for lack of virological cure in some studies can be inability of HCQ to reach the 50% effective concentrations (EC50) against SARS-CoV-2. Although dosage of HCQ chosen in most studies was comparable and enough to reach EC50, the fact remains that altered genome of

SARS-CoV-2 strains and/or host factors determining the drug’s pharmacokinetic or pharmaco-dynamic profile can have a strong bearing on treatment outcome. In fact, the role of genetics in determining blood HCQ concentrations and the need to consider individual cytochrome (particularly CYP2D6) polymorphisms before prescribing HCQ has been emphasized earlier.^[39]

No difference in unadjusted mortality rate with HCQ/CQ alone versus control was observed although when combined with AZ there was higher probability of death. The results were not significant for adjusted hazard ratios for HCQ/CQ as well as HCQ/CQ + AZ versus standard therapy. Due to “Very low” quality evidence for unadjusted MR, the results were not interpreted for drawing any conclusions. Adjusted HR results were more valid, as adjustment for confounding factors was done and GRADE generated “Moderate” quality evidence. Mahevas *et al.*^[27] presented results after adjustment for age, sex, comorbidities. Rosenberg *et al.* have adjusted for clinical findings like respiratory rate greater than 22 per min., O₂ saturation of less than 90%, abnormal chest imaging findings and comorbidities like DM, aspartate aminotransferase more than 40 U/L as these findings were more likely to be in patients receiving HCQ/CQ + AZ. Geleris *et al.*^[25] presented HR from multivariable Cox proportional model. Stratification was done for sex, chronic lung disease, body mass index. Additional adjustment for age, race, past diagnoses, ethnicity, drug treatment, vital parameters, etc., at baseline was done. Magangoli *et al.*^[26] and Yu *et al.*^[28] also presented HR adjusted for difference in baseline characteristics. Also, WHO Solidarity trial^[21] and recovery trial^[20] with large number of subjects (large dataset), showed no difference in mortality rates between HCQ and standard therapy, which is similar to conclusion of our meta-analysis.

In the study by Yu *et al.*, a lower dose of HCQ was used compared to other studies reporting higher mortality in HCQ treated groups. Yu *et al.* assumed that in critically ill COVID-19 patients having cytokine storm, HCQ mainly acts as anti-inflammatory and immuno-modulatory agent rather than as direct anti-viral and therefore HCQ was administered in lower anti-inflammatory doses. This was further confirmed by significant decline in IL-6 levels in HCQ group compared to control. The clinical potential of HCQ to limit acute inflammatory response was also highlighted in a clinical trial^[15,16] demonstrating encouraging results with respect to C-reactive protein levels and lymphocytopenia.

An inverse relationship between rate of disease progression and hospital discharge is usually expected which was also confirmed in our review as patients receiving standard of care treatment had a lesser probability of disease progression and higher probability of hospital discharge than those receiving HCQ/CQ. Although HCQ/CQ group exhibited increased odds of radiological improvement than control group, yet clinical significance of this finding might not be established due to its presence in small number of patients and in the absence of any clinically significant improvement over control group.

Table 2: GRADE recommendation for outcomes evaluated for the use of HCQ/CQ in patients with COVID-19 infection caused by SARS-CoV-2

No of studies; Study design	Certainty assessment					Effect		Certainty, Importance		
	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Efficacy Outcomes	Placebo		Relative (95% CI)	Absolute (95% CI)
Virological Cure (RT-PCR negative)										
6 RCT	serious ^{ab}	not serious ^c	not serious	not serious	none	293/340 (86.2%)	233/305 (76.4%)	OR 2.08 (1.36-3.17)	107 more per 1,000 (from 51 more to 147 more)	⊕⊕⊕⊕ MODERATE CRITICAL
Mortality										
10 OS; 5 RCT	serious ^e	serious ^f	not serious	serious ^g	none	840/4612 (18.2%)	1319/6422 (20.5%)	OR 0.98 (0.70-1.37)	3 fewer per 1,000 (from 52 fewer to 56 more)	⊕○○○ VERY LOW CRITICAL
Radiological Cure (CT scan)										
2 RCT	not serious	not serious	not serious	serious ^d	none	35/41 (85.4%)	26/43 (60.5%)	OR 3.89 (1.35-11.23)	251 more per 1,000 (from 69 more to 340 more)	⊕⊕⊕○ MODERATE IMPORTANT
Progression - ICU admission/Intubation/Severe Illness/CTI progression										
6 OS; 3 RCT	serious ^e	serious ^f	not serious	not serious	none	383/1646 (23.3%)	229/1627 (14.1%)	OR 1.77 (1.46-2.13)	84 more per 1,000 (from 52 more to 118 more)	⊕⊕○○ LOW CRITICAL
Hospital Discharge										
4 OS; 1 RCT	serious ^e	serious ^f	not serious	not serious	none	829/1234 (67.2%)	824/1104 (74.6%)	OR 0.64 (0.53-0.78)	93 fewer per 1,000 (from 137 fewer to 50 fewer)	⊕⊕○○ LOW IMPORTANT
Prolong QT interval										
3 OS	not serious ^h	not serious	not serious	not serious	strong association	49/347 (14.1%)	3/278 (1.1%)	OR 11.15 (3.95-31.44)	98 more per 1,000 (from 31 more to 245 more)	⊕⊕⊕⊕ HIGH CRITICAL
Mortality (HR)										
5 OS	not serious ⁱ	not serious ⁱ	not serious	serious ^g	none	-/0	-/0	HR 1.05 (0.86-1.29)	1 fewer per 1,000 (from 1 fewer to 1 fewer)	⊕⊕⊕○ MODERATE CRITICAL

CI: Confidence interval; OR: Odds ratio; RCT - Randomized control trials; OS - Observational study; †Huang et al. and Chen J et al. have some concerns with randomization concealment, which will affect the virological cure estimate, hence downgraded for quality of evidence. ‡Confounding in all three studies, along with missing data (Huang M et al) and selection of patients (Mallat et al) may affect the estimate. Studies have serious overall ROB, hence downgraded for evidence. †Although I² >40%, but exclusion of Mallat et al. (moderate selection bias) resulted in I²=0% without change in overall effect estimate. Hence heterogeneity ignored. †1. The Optimal Information Size (OIS) is 245 patients in each group, which was not met in the outcome. OR 2. Effect estimate 95% Confidence interval (CI) includes one. OR Both of the above reasons hence downgraded for Imprecision. †RCTs have a negligible contribution to the overall effect estimate and observational studies have moderate to serious ROB for unadjusted mortality rate, progression of disease, hospital discharge. Hence downgraded for overall ROB. †As I² >50%. Hence, Quality of evidence downgraded for heterogeneity. †As 95% CI includes one, hence downgraded for imprecision. †. Studies have low to moderate ROB, hence not downgraded. †. Adjusted Hazard ratios (HR) was presented by all the authors. Adjustment of all the covariates which might have led to the variability in mortality or cardiac arrest has been adjusted during analysis for mortality or cardiac arrest. Hence, not downgraded for risk of bias. †Although I² >40%, sensitivity analysis with exclusion of study done by Yu et al. resulted in I²=31% (<40%) without a change in overall mortality estimate. Only Yu et al. have concluded decrease mortality with the use of HCQ/CQ, hence heterogeneity was ignored

An important point to consider is time between symptom onset and treatment administration. Gautret *et al.*, in a non-randomized trial^[22] and cohort study,^[40] reported mean time as 4 ± 2.6 and 4.9 ± 3.6 days, respectively; with approximately 94% patients started with treatment within 2 days of symptom onset in the cohort study. In the study by Tang *et al.*,^[16] mean time from disease onset to randomization to treatment was 16.6 ± 10.5 days; the results were although consistent in subgroup analysis conducted on patients administered HCQ within 7 days of illness onset. Another study reported significant difference in period from onset of illness to treatment administration between two study groups [$6.5 (4.75-8.5)$ days in lopinavir/ritonavir vs $2.5 (2-3.75)$ days in CQ group; $P < 0.001$]; which probably could be a confounding factor for different outcomes in two groups.^[19] It may thus be hypothesized that early HCQ administration is likely to be associated with better clinical and virological outcomes in COVID-19 treatment.

Serious adverse event of concern with CQ/HCQ is QT interval prolongation with possible increased risk of torsades de pointes (TdP) and sudden cardiac death. Significant increase in odds of QT prolongation in patients administered HCQ/CQ alone or combined with AZ was found in our systematic review. For cardiac arrest, Rosenberg *et al.* concluded no increase in adjusted OR with HCQ/CQ alone but significant increase with HCQ/CQ + AZ. With reference to evidence from our systematic review, special attention should be paid to co-administration of other QTc prolonging drugs (azithromycin, antipsychotics, tricyclic antidepressants). In order to minimize the risk, guidelines by American College of Cardiology recommend withholding HCQ-AZ in patients with a QTc interval of 500 msec or greater at baseline.^[41]

Among other reported adverse events, most common were mild gastrointestinal effects (diarrhea, vomiting), rash, and headache. Blurring of vision was reported by 1 patient each in the studies by Tang *et al.*^[16] (1/70; 1.4%) and Gautret *et al.*^[22] (1/80; 1.2%). A higher dose of HCQ was used by Tang *et al.* (cumulative dose: 14.8--20.4 g of HCQ) as compared to other studies. Assuming less cumulative dose, which is the major risk factor for retinal toxicity with CQ/HCQ, we expect lesser chances of ocular toxicity in the setting of COVID-19 treatment. However, risk cannot be excluded entirely, as rapid onset of retinal toxicity following 1,000 mg daily dose of HCQ was reported in an earlier clinical trial.^[42] Therefore, standard monitoring guidelines need to be followed; according to American Academy of Ophthalmology, baseline screening tests including complete ophthalmologic examination and at least one newer, more sensitive test such as electro-retinogram, spectral domain optical coherence tomography, or fundus auto-fluorescence should be performed before starting CQ/HCQ therapy since the presence of retinal or macular disease is a relative contraindication to the use of these drugs.^[43] Probable lack of use of sensitive screening tests in the studies included in this review might be a reason for underestimation of retinal toxicity.

Limitations and strengths

One major limitation of current review is extraction of most of the data from 12 observational studies which have innate selection bias. Few not yet peer reviewed studies obtained from pre-print servers were also included. Major strengths of our review are inclusion of large datasets (WHO Solidarity and recovery trial) and GRADE analysis.

Conclusions drawn from current meta-analysis will play a major role in guiding the primary care physicians to make decisions with regard to COVID-19 management in community.

GRADE Pro analysis

Overall GRADE Pro was recommended as “Moderate” because important objectives such as adjusted MR had “Moderate” evidence. The critical outcomes like virological cure and radiological cure have “moderate” while QT prolongation have “high” quality of evidence. The outcomes like unadjusted MR and progression of disease were graded as “Very low” and “Low,” which implies that there is a high probability of future research having significant impact on our observations and is likely to change the estimate of effect. Hence, the results of both these outcomes were not given due consideration while drawing conclusions. Therefore, overall GRADE was recommended as “Moderate” quality evidence implying the potential of further research to have a bearing on the conclusions of this review.

Conclusion

Given the severity of disease and chaotic pandemic urgency, recommendations for treatment of COVID-19 are being made globally on the basis of insufficient evidence. The presence of sufficiently powered studies, moderate quality evidence generated for virological cure in favor of HCQ/CQ, and no difference in mortality carries conviction for its use in treatment of COVID-19 infection. However, safety concerns like QT prolongation, with high quality GRADE evidence raise enough red flags for random use of CQ/HCQ alone or co-administered with AZ in high-risk population for SARS-CoV-2. With current evidence, we recommend judicious and monitored use of HCQ/CQ in treatment of COVID-19 infection caused by SARS-CoV-2 in low to middle income countries with emphasis on lack of mortality benefit.

Ethics approval and consent to participate

Not a clinical trial, hence not applicable

Consent for publication

Done

Availability of data and material

All data is with authors.

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Conflicts of interest

There are no conflicts of interest.

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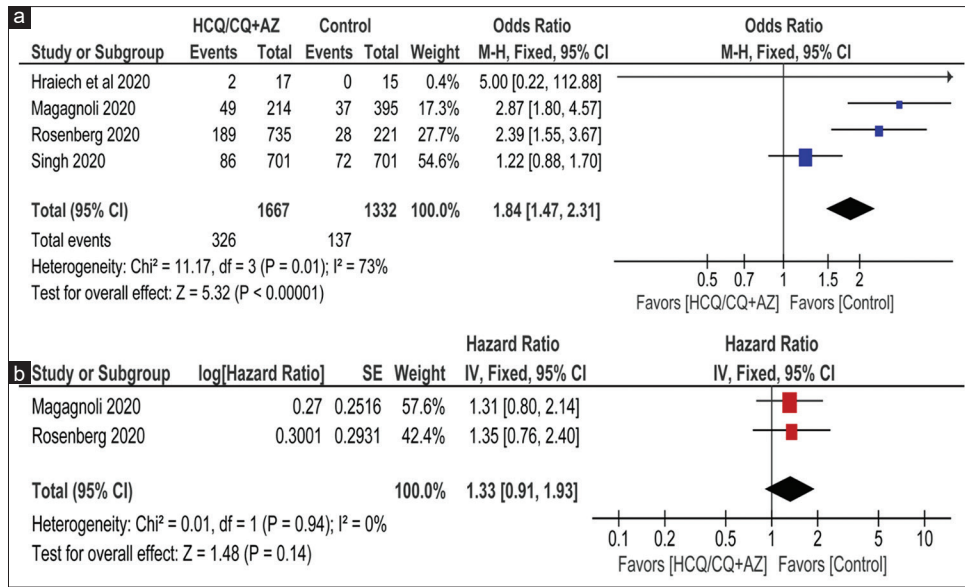


Figure 1S: Mortality rate (unadjusted OR-1Sa)(adjusted HR-1Sb) (HCQ/CQ + AZ vs control treatment)

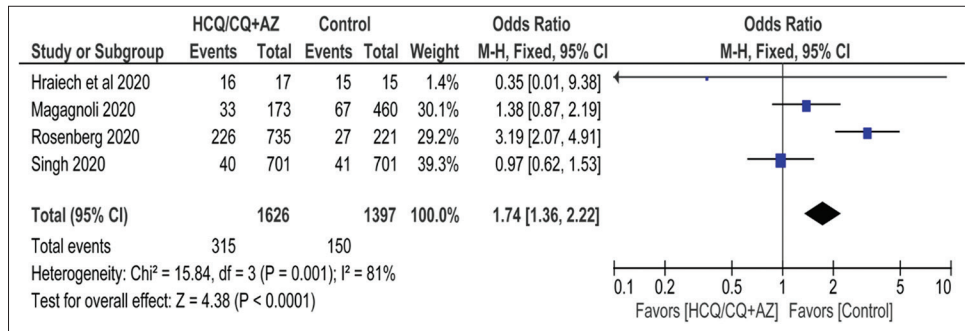


Figure 2S: Number of patients showing evidence of disease progression (HCQ/CQ + AZ vs control treatment)

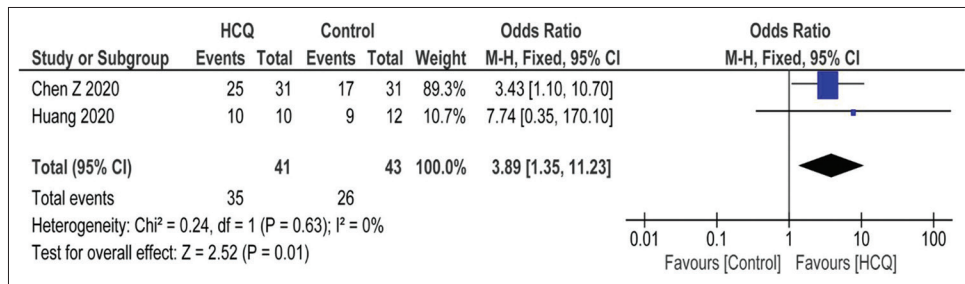


Figure 3S: Number of patients showing evidence of radiological cure (HCQ/CQ vs control treatment)

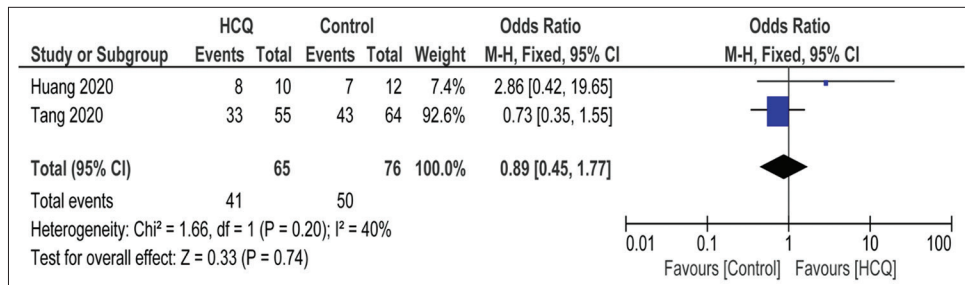


Figure 4S: Number of patients showing clinical improvement (HCQ/CQ vs control treatment)

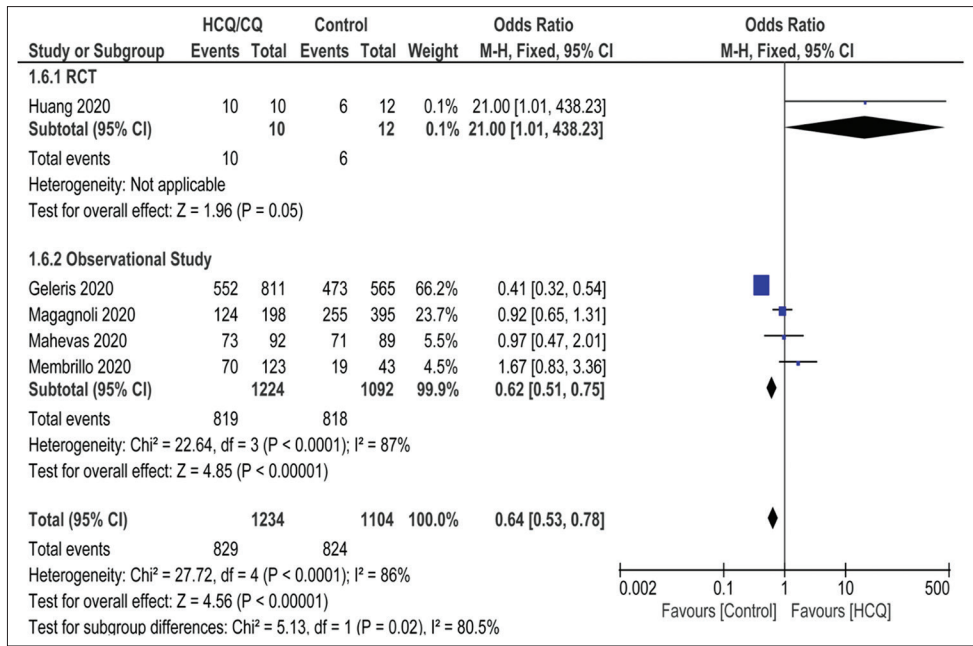


Figure 5S: Rate of hospital discharge (HCQ/CQ vs control treatment)

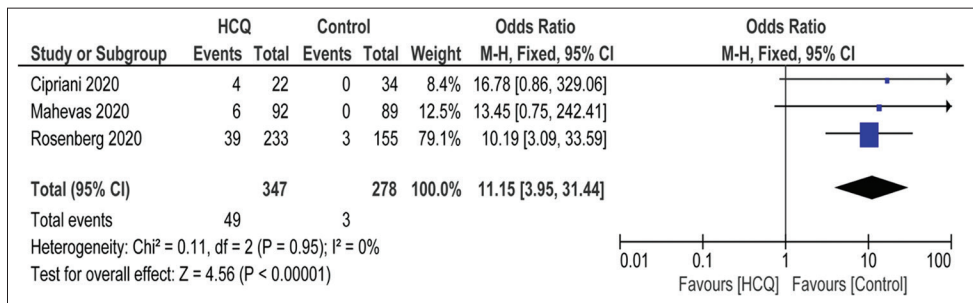


Figure 6S: QT prolongation (HCQ/CQ vs control treatment)

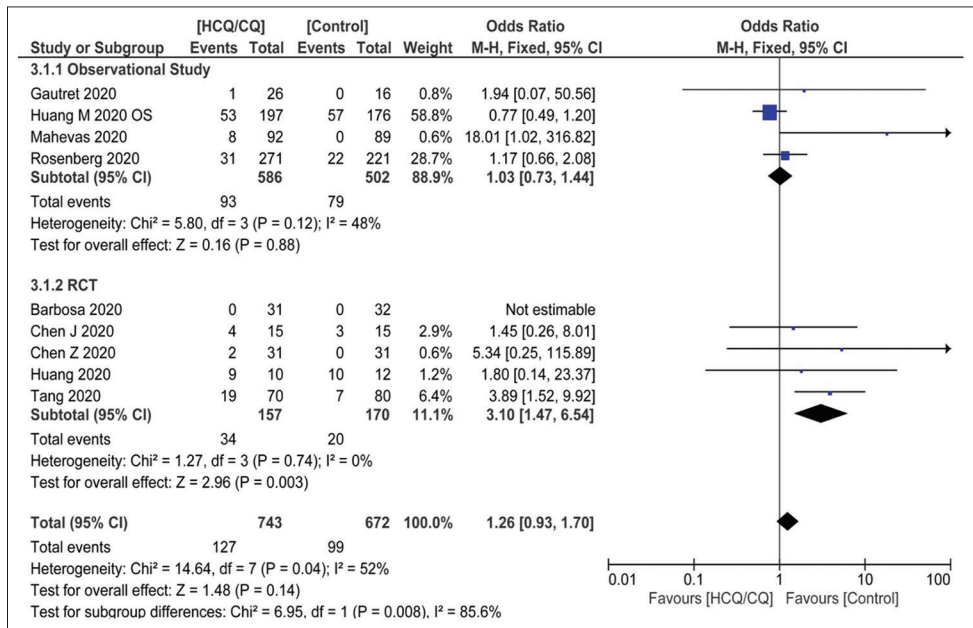


Figure 7S: Adverse events except QT prolongation and cardiac arrest or arrhythmias (HCQ/CQ vs control treatment)

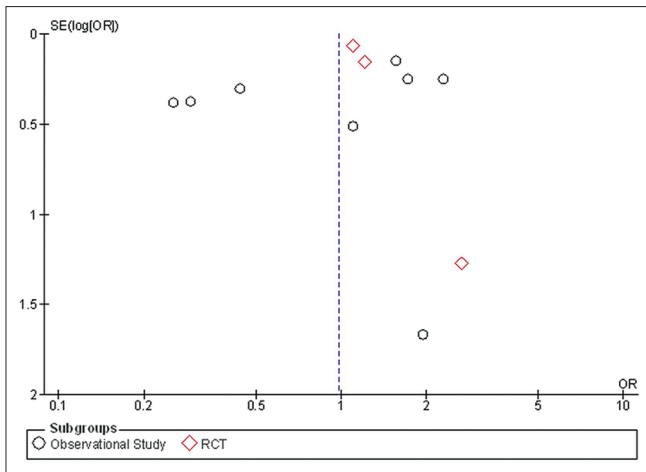


Figure 8S: Funnel plot depicting publication bias for studies included in the review