## **Letter to Editor**

# Chloroquine/Hydroxychloroquine and SARS-CoV 2; Lessons from Case-Control Studies

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ear Editor. In December 2019, a novel viral pneumonia caused by Severe Acute Respiratory Syndrome Coronavirus type 2 (SARS-CoV 2) was emerged in Wuhan, Hubei Province, China [1]. We are lives in global pandemic of coronavirus disease 2019 (COVID-19); However, we have limited therapeutic agents for treatment of COVID-19 and reduction of mortality caused by this virus; there is no FDA approved option against SARS-CoV 2 which is global concern [2].

According to review of the literatures, there are suggested several drugs including Remdesivir, Favi-piravir, Ribavirin, Interferons, Lopinavir/ritonavir, Oseltamivir, Chloroquine, hydroxy chloroquine, and azithromycin, Convalescent plasma, Herbal medications, non-steroidal anti-inflammatory drugs (NSAIDs), Myco phenolic acid, Monoclonal or polyclonal anti-bodies, and, Angiotensin-converting enzyme 2 genebased peptides for combating with SARS-CoV 2 [3]; but there is no conclusive evidence for efficacy and safety of these therapeutic agents against COVID-19 [4-5].

The Lopinavir/ritonavir was initially recommended for treatment of COVID-19; but Cao et al., 2020 showed that there is no clinical benefit in COVID-19 cases which are received lopinavir-ritonavir and control group [5-6].

Subsequently, it's suggested that Chloroquine/ Hydroxychloroquine can be used against SARS-CoV 2 (2-4). Chloroquine (CQ) has several benefits such inhibitory effects in SARS-CoV-2 infected Vero-E6 cell lines (EC50 =  $1.13~\mu M$ ), increasing endosomal pH, dysregulating the glycosylation of angiotensin-converting enzyme 2 receptors, immunomodulatory activity, provoke T regulatory cells, as well as, clinically beneficial effect in recently clinical trials which is conducted by Huang et al., 2020 (2-3,6).

Moreover, there is concern about CQ due to its toxicity and Hydroxychloroquine (HCQ) was introduced as alternative option of CQ; particularly, HCQ has inhibitory roles in SARS-CoV infected Vero cells  $(0.72 \mu M)$  [7].

Therefore, CQ/HCQ has emerged as potential therapeutic option against SARS-CoV 2; there are numerous registered clinical trial which are evaluating the clinical benefit of CQ/HCQ for treatment of COVID-19.

Herein, the aim of study was evaluation of clinically benefits of CQ/HCQ for treatment of COVID-19 using statistical analysis of provided evidence. We conducted a systematic search in several databases including PubMed, Scopus, Embase, EBSCO, Google scholar, Cochrane library, medRxiv, and bioRxiv to retrieving all available case-control articles in relation to efficacy of CQ/HCQ in treatment of SARS-CoV 2. we have no limit in language or date in searching and data collection; also, we used from several keywords according to MeSH including "2019-nCoV", "2019 novel coronavirus", "COVID-19", "coronavirus disease

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2019", "chloroquine", "hydroxychloroquine", and "Plaquenil" for searching databases. In the next, titles, abstracts of all obtained studies were screened to remove duplicates and collected all relevant case-control studies. Subsequently, the full-text of relevant studies were evaluated carefully and required data were extracted in the Table 1. Finally, clinical benefit of CQ/HCQ was measured with odds ratio with 95% confidence intervals.

The patient status was mild to severe requiring O2. This studies were conducted in France, China, USA, Spain, and UAE.

According to statistical analysis, there is no significant benefit in clinical improvement of COVID-19 patients were received CQ/HCQ in comparison with control (OR:1.089; 0.82-1.432 with 95% CIs; p-Value: 0.54; I2: 58.97; Q-Value: 9.75; p-Value: 0.045; Eggers p-Value: 0.51; Beggs p-Value: 0.04).

| Table 1. Characteristics of included studies |               |         |      |                 |         |                                 |                 |             |         |                 |         |        |                      |      |         |    |
|--|---------------|---------|------|-----------------|---------|---------------------------------|-----------------|-------------|---------|-----------------|---------|--------|----------------------|------|---------|----|
| Study  | Study type    | Country | Age  | No. of patients |         | Severity of disease             | HCQ /CQ<br>dose | Improvement |         | Viral clearance |         | Death  | Severity progression |      | Ref     |    |
|  |               |         |      | Case            | Control |                                 |                 | Case        | Control | Case            | Control | Case   | Control              | Case | Control |    |
| Gautret                                      | nRCT          | France  | 45.1 | 20              | 16      | Moderate                        | 600 mg/d        | NR          | NR      | 14              | 2       | NR     | NR                   | NR   | NR      | 8  |
| Mahevas                                      | Retrospective | France  | 60   | 84              | 97      | Moderate/Pneumonia requiring O2 | 600 mg/d        | NR          | NR      | NR              | NR      | 2.8%   | 4.6%                 | 24   | 23      | 9  |
| Mallat                                       | prospective   | UAE     | 37   | 23              | 11      | Moderate/Pneumonia requiring O2 | 400 mg/d        | NR          | NR      | 47.8%           | 90.9%   | NR     | NR                   | NR   | NR      | 10 |
| Tang   | RCT           | China   | 46   | 75              | 75      | Moderate                        | 1200<br>mg/d    | 59.9%       | 66.6%   | 85.4%           | 81.3%   | NR     | NR                   | NR   | NR      | 11 |
| Jun  | RCT           | China   | 30   | 15              | 15      | Moderate                        | 400 mg/d        | 15          | 15      | 13              | 14      | NR     | NR                   | 5    | 7       | 12 |
| Chen   | RCT           | China   | 44.7 | 31              | 31      | Moderate                        | 400 mg/d        | 25          | 17      | NR              | NR      | NR     | NR                   | 0    | 4       | 13 |
| Rosenberg                                    | Retrospective | USA     | NR   | 271             | 431     | Moderate                        | 400 mg/d        | NR          | NR      | NR              | NR      | 54/271 | 21/211               | 52   | 50      | 14 |
| Huang  | RCT           | China   | 44   | 10              | 12      | Moderate                        | 400 mg/d        | 60%         | 25%     | 10              | 12      | NR     | NR                   | NR   | NR      | 6  |
| Geleris                                      | RCT           | USA     | NR   | 811             | 565     | Mild/Moderate                   | 400 mg/d        | NR          | NR      | 65.0%           | 18.8%   | 262    | 84                   | NR   | NR      | 15 |
| Huang  | Prospective   | China   | 43   | 197             | 176     | Mild/Sever                      | 500 mg/d        | NR          | NR      | 95.9%           | 79.6%   | NR     | NR                   | NR   | NR      | 16 |
| Barbosa                                      | qRCT          | USA     | 62.7 | 32              | 31      | Moderate                        | 800 mg/d        | NR          | NR      | NR              | NR      | 6      | 2                    | NR   | NR      | 17 |
| Magagnoli                                    | Retrospective | USA     | 68   | 198             | 395     | Moderate                        | NR              | 124         | 255     | NR              | NR      | 38     | 37                   | NR   | NR      | 18 |
| Membrillo                                    | Retrospective | Spain   | 51.5 | 123             | 43      | Mild/Sever                      | 800 mg/d        | 70          | 19      | NR              | NR      | 27     | 21                   | NR   | NR      | 19 |
| Yu   | Retrospective | China   | 68   | 48              | 502     | Mild/Sever                      | 400 mg/d        | NR          | NR      | NR              | NR      | 9      | 238                  | NR   | NR      | 20 |

We analyzed the efficacy of CQ/HCQ in 1) clinically improvement of COVID-19 patients, 2) SARS-CoV 2 clearance (by PCR), 3) reduction of death, and 4) prevention form progression of disease severity. All statistical analysis was conducted using Comprehensive Meta-Analysis (CMA) software version 2.2 (Biostat, Englewood, NJ, USA). The pooled analysis was measured by fixed-effect models; However, in high heterogeneity cases, I2 index>25% and Cochrane Q test p-value ≤0.05, we used from random effect model based on Dersimonian and Laried method. In addition, publication bias was assessed by Eggers p-Value and Beggs p-Value.

We provided 14 relevant case-control studies (total participate: 4,338) in regarding to evaluation of CQ/HCQ beneficial effects in treatment of COVID-19. The study types were RCT, nRCT, qRCT, retrospective, and prospective. In the present report, there is 1,938 SARS-CoV 2 infected cases which received CQ/HCQ as well as 2,400 patients as control (not received any dose of CQ/HCQ). The mean age of patients was estimated about 50.2 years.

In addition, CQ/HCQ administration has not preventive effects on mortality due to COVID-19 (OR: 2.03; 1.66-2.47; p-Value: 0.001; I2: 91.86; Q-Value: 73.72; p-Value: 0.001; Egers p-Value: 0.13; Beggs p-Value: 0.18). However; there is significant increasing of viral clearance in CQ/HCQ treated cases (OR:6.50; 5.17-8.19; p-Value: 0.001; I2: 85.74; Q-Value: 35.0; p-Value: 0.001; Eggers p-Value: 0.06; Beggs p-Value: 0.12). Therefore, Although CQ/HCQ was efficient in SARS-CoV 2 virological cure; but there is no efficient in recovery and improvement of patients. Also, CO/HCO has no preventive effect on mortality of COVID-19 patients (Hazard Ratio: 1.073; 0.89-1.29 with 95% CIs; p-Value: 0.46; I2: 72.15; Q-Value: 14.36; p-Value: 0.006; Eggers p-Value: 0.38; Beggs p-Value: 0.50). Unfortunately, we found that disease severity (transfer to ICU; radiological chest progression, require to mechanical ventilation, and develop into acute respiratory distress syndrome) was significantly higher among COVID-19 patients were received CQ/HCQ which is represent inefficacy of these drugs in treatment of COVID-19 (OR: 1.49; 1.05-2.10; p-Value: 0.023; I2: 48.92; Q-Value: 5.87; p-Value: 0.11; Eggers p-Value: 0.001; Beggs p-Value: 0.044).

Chloroquine has several disadvantages consisting hypoglycemia, diarrhea, prolong QTc interval, and AV block [21-22]. In addition, Gendelman et al., recently published an article about inefficacy of continuous Hydroxychloroquine or colchicine therapy for prevention of SARS-CoV 2 infection [23]. According to recent RCTs, HCQ has no therapeutic effects on prevention or improvment of major outcomes in COVID-19 patients [24-25]. According to current analysis, it seems that CQ/HCQ has not benefit in treatment of COVID-19 patients. But we have several limitations such as 1) high heterogeneity rate which reduce reliability of analysis, 2) presence of publication bias, 3) dosage of CQ/HCQ was varying among the included studies, 4) different outcome between studies, 5) various in times of evaluating outcomes, and 6) low sample size. We need to complete further clinical trials to confirmed our results.

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## **Conflict of interest**

The authors have no conflict of interest.

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