Clinical Medicine & Research
Volume 19, Number 4: 179-182

clinmedres.org

Original Research

Effect of Chloroquine and Hydroxychloroquine on Cytokine Release Syndrome in Patients with COVID-19

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Objective: To assess the effect of chloroquine and hydroxychloroquine on cytokine release syndrome (CRS) in adult patients with coronavirus disease 2019 (COVID-19) having mild to moderate symptoms.

Methods: This blinded, placebo-controlled, randomized study was conducted in the Department of Medicine, Pak Emirates Military Hospital Rawalpindi, from June 1–15, 2020. A total of 150 hospitalized patients were enrolled after diagnoses with COVID-19 through reverse transcription polymerase chain reaction (RT-PCR). They were divided into three groups: hydroxychloroquine plus general care (HGC, n=50), chloroquine plus general care (CGC, n=50); and only general care (OGC, n=50). The HGC group received treatment with hydroxychloroquine 400 mg every 12 hours for day one and 200 mg for the next 4 days. The CGC group received treatment with chloroquine 250 mg every 12 hours for 7 days. The OGC group was kept as a control with only general care. After 12 days, the patients were screened for development of CRS through detection of interleukin 6 (IL-6) in serum samples by using Roche cobas e411 electrochemiluminescence immunoassay analyzer.

Results: The mean duration from onset of symptoms to randomization was 7.65 days (SD = 3.287 days; range, 2-15 days). The mean age of patients was 37.57 (range 19-63) years. Results showed that out of a total 150 patients, only 10 patients (6%, mean=1.93; CI=1.89-1.97, *P*=0.651) developed CRS in all study groups. Four patients (8%) developed CRS in the HGC group, 2 patients (4%) in the CGC group, and 4 patients (8%) in the OGC group. There was no significant difference in the mean level of CRS among study groups.

Conclusion: Administration of hydroxychloroquine and chloroquine has no effect in reducing the development of CRS in patients with COVID-19 having mild to moderate symptoms.

Keywords: Chloroquine; Coronavirus disease; COVID-19, Cytokine Release Syndrome; Hydroxychloroquine

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Disclosures: The authors have reported no conflicts of interest or financial support for this work.

Received: January 11, 2021
Revised: May 3, 2021
Accepted: June 23, 2021

doi:10.3121/cmr.2021.1659

The first case of coronavirus disease-19 (COVID-19) was reported in December 2019, in Wuhan, China. COVID-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and is zoonotic in origin. After its rapid spread to other countries, it was declared a pandemic on March 12, 2020 by the World Health Organization (WHO) within months of initial reports from China. The disease usually has mild symptoms, but almost 5% of cases have complications such as severe respiratory failure or multi organ dysfunction.[1] These complications may include cytokine release syndrome (CRS), which is characterized by fever, elevation of inflammatory markers, hyperferritinemia, and thrombocytopenia.[2] Globally, as declared by WHO on July 20, 2020, there were 14,348,858 confirmed cases of COVID-19 with a total of 603,691 deaths. In the Eastern Mediterranean, there were a total of 1,387,295 confirmed as of July 2020. Similarly in Pakistan, there were 265,083 confirmed cases of COVID-19, with 5599 deaths, as of July 20, 2020. Out of those, 205,929 cases had recovered, while 1552 were critically ill.[4]

The pandemic has been challenging due to a lack of conventional drugs and treatment protocols. With the rapid spread of disease, even the off-label use of conventional drugs has issues regarding the availability of the drugs. Until recently, antiviral drug therapy has been of doubtful effectiveness, and even with the vaccine, there are varying levels of immunization. The available treatment is also limited to basic supportive care and ventilator support for complicated cases.[5] Presently no effective drugs are available to fight against COVID-19, whereas a few antimalarials (chloroquine and hydroxychloroquine) and antivirals (remdesivir, favipiravir) have been put forward as possible therapies. These drugs have already shown promising results against other coronavirus diseases, like Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory syndrome (SARS).[6] Although, these drugs have not yet shown any clinical results in the treatment of COVID-19. Additionally, in a recent study conducted on hospitalized adult patients with severe disease symptoms, lopinavir-ritonavir has not shown any beneficial results.[7] Chloroquine and hydroxychloroquine are approved anti-malarial drugs; however, they are being used and have shown promising effects in certain autoimmune diseases in the fields of rheumatology and dermatology. The mode of action of antimalarials is still unclear in autoimmune diseases, but it may include anti-inflammatory effects along with immunomodulation on the host. Off-label usage of chloroquine and hydroxychloroquine was first established in the SARS epidemic in Asia during 2002 and 2003. During the COVID-19 pandemic, research did show some promising results of chloroquine and hydroxychloroquine including on inhibition of viral entry and later stages of infection after viral entry into a cell.[8]

Hydroxychloroquine treatment is quite safe; side effects usually depend on dose and duration. Dermatological, gastrointestinal, and neurological side effects are the most common, while retinal and cardiac side effects (long QT interval) are less common but carry significant morbidity.[9] Chloroquine and hydroxychloroquine are being used in the treatment of COVID-19 as per Chinese guidelines,[10] and the United States Food and Drug Administration (FDA) has also approved their use in the pandemic.[11] However, there has been no promising evidence from clinical trials that gives acceptable results for the use of chloroquine or hydroxychloroquine in the treatment of COVID-19. In this pandemic, health care workers are overburdened, over worked, and are at risk of exposure to COVID-19 due to lack of exact treatment.[7] Therefore, we conducted this randomized, controlled study to look at the effect of hydroxychloroquine and chloroquine in adult patients with COVID-19.

Methods

Study Design and Ethical Approval

This single-blinded, placebo-control study was conducted at Pak Emirates Military Hospital, Rawalpindi from June 1-15, 2020 after ethical approval from Hospital Ethical Committee.

Inclusion and Exclusion Criteria

Patients who were admitted into the hospital with COVID confirmed by PCR and having mild to moderate symptoms as per Chinese guidelines for COVID-19 were chosen for enrollment. The age range of the patients was 19 to 63 years. Exclusion criteria were patients with severe or critical COVID-19, pregnant women, patients with ischemic heart disease or cardiac arrhythmias, retinopathy, and patients who refused to participate in the study.

Allocation of Study Groups and Protocol

A total of 150 patients, both male and female, were recruited and signed an informed consent form. They were randomly allocated to one of three study groups: hydroxychloroquine plus general care (HGC); chloroquine plus general care (CGC); and only general care (OGC). Each study group consisted of 50 patients. The HGC group received treatment with hydroxychloroquine 400 mg every 12 hours for day one and 200 mg for the next 4 days and were also provided general care. The CGC group received treatment with chloroquine 250 mg every 12 hours for 7 days and were also provided general care. The OGC group was kept as the control and were provided with only general care. After 12 days, the patients were screened for development of CRS through detection of interleukin 6 (IL-6) in blood samples.

Determination of CRS

A 5 mL venous blood sample was collected from each patient and was centrifuged at 3500 RPM for 10 minutes. Serum specimens were stored at -70°C in aliquots to avoid possible interference with assay results due to repeated freeze-thaw cycles. Serum IL-6 concentration was determined by commercially available high sensitivity indirect sandwich enzyme-linked immunosorbent assay (Bender MedSystems,

Austria). Briefly, IL-6 present in the samples or standard binds to anti-IL-6 monoclonal antibody adsorbed to the microwells. A biotin-conjugated monoclonal anti-IL-6 antibody was added and binds to IL-6 captured by the first antibody. Following incubation, unbound biotin-conjugated anti-IL-6 is removed during a wash step. Streptavidin-horseradish peroxidase (HRP) was added and binds to the biotin-conjugated anti-IL-6; following incubation unbound Streptavidin-HRP was removed during a wash step, and substrate solution reactive with HRP was added to the wells. A colored product was formed in proportion to the amount of IL-6 present in the sample. The reaction was terminated by addition of acid, and absorbance was measured at 450 nm. A standard curve was prepared from seven IL-6 standard dilutions and IL-6 sample concentrations determined.

Statistical Analysis

Data were analyzed with SPSS version 24.0. The three groups were compared for all the analyzed tests after the treatment. All of the quantitative variables (CRS) were described as Mean ± SD and frequency (%), respectively. Differences in the characteristics between the groups were compared using the Mann-Whitney test and *t*-test as appropriate. A *P* value of <0.05 was considered statistically significant.

Results

This study was carried out to determine whether either consumption of hydroxychloroquine and chloroquine helps in reduction of CRS development in patients with COVID-19. After an average of 7 days of the treatment, the determination of IL-6 in serum was used as an indicator for the development of CRS. Results showed that out of a total of 150 patients, only 10 patients (6%, mean=1.93; SD= 0.25, CI=1.89-1.97, *P*=0.651) developed CRS in all study groups. The mean duration from onset of symptoms till randomization was 7.65 days (SD=3.287; range, 2-15 days). The mean age of patients was 37.57 years (range 19-63 years). Of all patients, 143 were male (95.3%), and only 7 were females (4.6%). In the HGC group, four patients (8%, mean=1.92, SD=0.274) developed CRS. In the CGC group, two patients (4%, mean=1.96, SD=1.98) developed CRS; in the OGC group four patients (8%, mean=1.92, SD=0.274) developed CRS. However, the results demonstrated that overall only 6% of the patients (n=10) developed CRS in COVID-19 infection. This indicated that treatment with hydroxychloroquine and chloroquine might assist general care during COVID-19 infection to reduce the progression of CRS, although there was no significant difference in the mean value of CRS among the three groups.

Discussion

The study clearly shows there is no strong evidence to justify the use of chloroquine or hydroxychloroquine in preventing the development of CRS in patients with mild to moderate COVID-19 infection. Our results showed that out of a total of 150 patients, 10 patients (6%, mean=1.93; SD= 0.25, CI=1.89-1.97, *P*=0.651) developed CRS in all study groups. The mean duration from onset of symptoms till randomization was 7.65 days. The mean age of patients was 37.57 years. A study conducted in China by Tang et al[12] in May 2020 was the first randomized controlled trial, and their results were almost similar to our study. They used hydroxychloroquine compared with standard of care alone in 150 PCR positive COVID-19 patients. This study showed the probability of negative sero-conversion by 28 days in the standard of care plus hydroxychloroquine group was 85.4% (95% CI, 73.8%-93.8%), similar to that in the standard of care group (81.3%, 71.2%-89.6%). The difference between these two groups was 4.1%.[13]

Chen et al[14] conducted a pilot study at Shangai Public Health Centre to evaluate the efficacy of hydroxychloroquine in patients with moderate COVID-19. They divided 30 patients into two groups: a control group and a hydroxychloroquine group. The end result of the study was negative conversion rate of SARS-CoV-2 nucleic acid in pharyngeal swab on day 7. The results showed a negative throat swab in 13 cases (86.7%) in the hydroxychloroquine group and 14 cases (93.3%) in the control group. The median duration for negative seroconversion was 4 days in the hydroxychloroquine group and 2 days in the control group. Their study correlates with our study in which hydroxychloroquine has not produced positive results in patients with COVID-19. Their study went further than ours, as they included negative seroconversion in their end result.[14]

The finding of a clinical trial by Self et al[15] showed that hydroxychloroquine was not efficacious for the treatment of COVID-19 in hospitalized patients, which is consistent with our results. This multicenter placebo-control trial was conducted at 34 hospitals in the United States. The 479 patients with confirmed COVID-19 were randomly assigned to either a hydroxychloroquine group or a placebo group. The outcome of their study was clinical status after 14 days, ranging from 0 (ie, death) to 7 (ie, discharge from hospital and resuming daily normal activities). The results were not significantly different between the hydroxychloroquine group and the placebo group (median [IQR] score, 6 [4-7] vs 6 [4-7]; OR, 1.02 [95%CI, 0.73 to 1.42]).[15] Similarly, in our study the outcome was the development of CRS, which showed no significant results in all three groups of our study.

There were certain study limitations to our study, including that the IL-6 level alone may not be sufficient to reflect its functional downstream effects; an assay that distinguishes functional IL-6 from total IL-6 may provide a refined approach to guide therapeutic decisions. C-reactive protein (CRP), an acute-phase inflammatory protein synthesized by IL-6-dependent hepatic biosynthesis, is a reliable marker of IL-6 bioactivity and is used to predict CRS severity and monitor IL-6 blockade efficacy.

Conclusion

Administration of hydroxychloroquine and chloroquine has no effect in reducing the development of CRS in patients with COVID-19 having mild to moderate symptoms. The study also clearly showed no strong evidence for the efficacy of hydroxychloroquine or chloroquine in the prevention of CRS. Further large scale clinical trials are needed to explore the efficacy of these drugs on CRS development with corona virus infections.

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