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Background

There is an urgent need for an effective treatment to cure patients with COVID-19 and reduce the duration of viral shedding.

Methods

We conducted a retrospective study using data from the electronic medical records of patients with confirmed SARS-CoV-2 infection who were hospitalized in the E1-4 intensive care center of Guanggu Hospital, Wuhan City, Hubei Province, China, from February 11, 2020, to March 23, 2020. According to the diagnostic results, the hospitalized patients were divided into the experimental group treated with hydroxychloroquine (HCQ) or chloroquine (CQ) and the control group only treated with conventional therapy without HCQ or CQ. The main outcome was the clearance rate of SARS-CoV-2.

Results

A total of 37 patients were evaluated. Eighteen patients were assigned to the HCQ or CQ group, and 19 were assigned to the routine treatment group. Treatment with HCQ or CQ was not associated with a difference from routine treatment in the viral shedding duration (median, 14 days vs. 10 days; hazard ratio for viral shedding, 0.393; 95% confidence interval [CI], 0.151 to 1.022; P=0.056). No significant difference in the viral shedding rate was observed between the groups at any time point (7 days, 14 days, 21 days, 28 days and the end point).

Conclusion

Although this is a retrospective analysis, the results suggest that treatment with HCQ or CQ had no impact on the duration of viral shedding.

Keywords: SARS-CoV-2, COVID-19, Hydroxychloroquine, Chloroquine

Introduction

In December 2019, a series of “unknown pneumonia cases” were found in Wuhan, Hubei Province, China. The illness was termed novel coronavirus disease (COVID-19) and spread rapidly worldwide, becoming an urgent concern for the international community[1]. The main symptoms of COVID-19 include fever, cough, chest pain, dyspnea, fatigue, muscle soreness and gastrointestinal symptoms, and the disease can be transmitted from person to person[2]. By June 26, 2020, more than 9.7 million cases of COVID-19 had been reported worldwide, and the total number of deaths has exceeded 490000. This has brought tremendous health and economic burdens to people around the world. In this emergency situation, it is very important to find effective treatment protocols and stop the spread of the epidemic, but no "wonder drug" has been found. Among the potential solutions currently being researched, HCQ

or CQ has received special attention for the treatment of novel coronavirus disease[3]. Chloroquine has been prescribed extensively for the prevention and treatment of malaria as well as the treatment of autoimmune conditions[4, 5]. Later, safer hydroxychloroquine was discovered. In addition to activity against rheumatic diseases, the two antimalarial agents have also shown therapeutic activity or immune modulatory effects in other diseases, including antiphospholipid syndrome, amebiasis, HIV/AIDS, and some cancers. Here, we report a retrospective evaluation of 58 COVID-19 patients treated for 5 days with HCQ or CQ during hospitalization, with a follow-up period of at least 6 days. The outcome was the persistence of viral shedding.

Methods

The study was conducted in Guanggu Hospital in Wuhan, Hubei Province, China. This hospital was one of the main hospitals designated for treating patients with new coronavirus infections during a specific period. Fifty-eight patients diagnosed with COVID-19 with positive nasopharyngeal or oropharyngeal swab samples before admission were admitted to the hospital and stayed in the E1-4 intensive care center during the period from February 11 to March 23, 2020. A guideline developed by the National Health Commission of the People's Republic of China and distributed to all internal staff of our medical center suggests that HCQ or CQ can be a treatment option for severe respiratory diseases related to COVID-19.

After 10 days of routine treatment, 20 patients were found to have viral shedding, and 1 patient died within 5 days of admission; we excluded these patients from the study. Ultimately, we obtained 18 patients in the HCQ or CQ group and 19 in the routine treatment group (Figure 1).

From the clinical database, we obtained the following data in the first review of the records for each patient: age, sex, respiratory rate at admission, previous medical history, relevant test results, etc. Other symptoms except fever and the date of symptom onset, among others, were estimated with the use of methods developed by our team. Virological follow-up included ≥1 test(s) performed systematically on days 2, 6 and 10. Patients with persistent positive PCR results on day 10 were further tested at least every 3 days until the test results were negative three consecutive times.

HCQ or CQ Exposure and Grouping

If a patient was treated with HCQ or CQ during hospitalization, he or she was classified into the experimental group. The control group consisted of the other patients who did not receive HCQ or CQ. The experimental group received routine treatment and oral administration of hydroxychloroquine 400 mg twice a day for 5 days or chloroquine 500 mg twice a day for 7 days. The control group only received routine

treatment, including bed rest, inhaled oxygen supplementation, and symptomatic supportive treatment, using the antiviral drugs recommended in the "diagnosis and treatment plan" (http://www.nhc.gov.cn/yzygj/s7653p/202002/8334a8326dd94d329df351d7da8aefc2/files/b218cfeb1bc54639af227f922bf6b817.pdf), such as interferon α atomization, oral administration of Arbidol or lopinavir/ritonavir, traditional Chinese medicine, and appropriate antimicrobial treatment if necessary.

Outcome

The primary study end point was viral clearance as determined by SARS-CoV-2 negative pharyngeal swabs, sputum, or lower respiratory tract secretions. The reported SARS-CoV-2 nucleic acid test result of the respiratory specimens is based on the last test result and time. If the test was negative for viral nucleic acid for multiple consecutive times, the first time when the nucleic acid test result was negative was used. If a positive result occurred again after turning negative, the negative result will not be used.

Statistical Analysis

The quantitative variables within each group were described using means, medians and standard deviations. In addition, the Shapiro-Wilk normality test was applied. For variables with normal distribution in the two groups, Student's t test was used to compare groups; otherwise, the Mann-Whitney test was used. The duration of viral shedding was portrayed by a Kaplan-Meier plot and compared with a log-rank test. Hazard ratios with 95% confidence intervals were calculated by means of the Cox proportional hazards model. The software used in the analysis was SPSS version 26.0.

Ethics statement

The protocol was approved by the ethics committee of Guanggu Hospital, Wuhan City.

Results

The median age of the patients was 60 years old (interquartile range [IQR], 43.5 to 67.0 years), and 43.2% of the patients were men (Table 1). There were no relevant differences between groups in terms of demographic characteristics, baseline laboratory test results, clinical symptoms, or underlying diseases. During the study, systemic Zadaxin was administered to 55.6% of the patients in the experimental group and to 26.3% of those in the control group.

Patients assigned to HCQ or CQ were similar to patients in the control group with respect to the duration of viral shedding (median, 14 days vs. 10 days; hazard ratio for viral shedding, 0.393; 95% confidence interval [CI], 0.151 to 1.022; P=0.056) (Figure 2). No significant difference was observed among the patients in terms of the rate of viral shedding at any time point (7 days, 14 days, 21 days, 28 days and the end point) (Table 2).

Safety

There were 2 mild gastrointestinal adverse events in the experimental group, but there were no adverse events in the control group. Both adverse events were judged by the investigators to be related to the medication.

Discussion

The novel coronavirus disease (COVID-19) is a high mortality epidemic disease with no specific treatment. Medical workers in various countries are looking for effective treatment methods. Our analysis is not a randomized trial but is based on the real experience of doctors who treat patients during the pandemic. The National Health Committee of the People's Republic of China issued a "diagnosis and treatment plan" at the early stage of the COVID-19 epidemic, in which hydroxychloroquine and chloroquine were recommended as one of the antiviral treatment regimens for COVID-19. However, the therapeutic effect of hydroxychloroquine and chloroquine against COVID-19 has become a controversial issue in the medical field. Evidence is needed on patients with COVID-19 who are treated with such drugs.

Several clinical studies addressing the efficacy of HCQ for the treatment of COVID-19 reported contradictory results. Four studies showed that HCQ had a favorable effect in terms of improving the clinical efficacy among COVID-19 patients[6-9]. However, some studies have reported different conclusions[10-12]. All the patients in this article have severe disease, and the results show that the experimental group did not show clinical efficacy of the treatments in accelerating viral clearance. We are also looking forward to the conclusions of early intervention studies, such as prophylactic treatment with azithromycin and hydroxychloroquine in hospitalized patients with COVID-19 (ProPAC-COVID)[13].

COVID-19 patients in our center are all seriously ill. We evaluated patients who received treatment for at least 14 days in isolation. The patients were seriously ill when they were admitted to the hospital. However, the proportion of patients who deteriorated after treatment was extremely low because no patients were transferred to the intensive care unit. In addition, it is worth noting that half of the patients (29/58) in this study had viral shedding 14 days after admission. Most importantly, only 1 patient died during the observation period.

This analysis involves a small sample of consecutive patients who had been hospitalized with COVID-19. Patients who received HCQ or CQ treatment had not significantly longer viral shedding times than patients who did not ($P>0.05$). In the 37 patients, we found that, compared with standard supportive care alone, the addition of HCQ or CQ treatment did not reduce the duration of viral RNA detection. A total of 29.3% of patients in the experimental group were found to have detectable SARS-CoV-2 RNA at the end of the study (day 40). Recently, a retrospective analysis of 1061 cases in Marseille, France, showed that early treatment of 973 (91.7%) COVID-19 patients with hydroxychloroquine and azithromycin demonstrated good

clinical outcomes and virological cures[14]. Perhaps we treated the patients with HCQ or CQ too late in our study. Given the observational design, this study does not determine the benefits or harms of HCQ or CQ treatment. However, our results are in line with the latest recommendations and do not support the use of hydroxychloroquine.

Our study has some limitations. First, as the study was not blinded, it is possible that the knowledge of the treatment assignment might have influenced clinical decision-making, which could have affected the viral shedding that was our main end point. We will continue to follow these patients to evaluate their long-term prognosis. Second, due to the overwhelmed health services system, the data for some patients were incomplete. For example, serum drug levels were not available for all patients, notably in those admitted during nonbusiness hours, and routine CT scans of some patients were not assessed. As this study was observational, the study results should not be taken to rule out the benefits or harms brought about by HCQ or CQ treatment.

Overall, based on our experience, we believe that we can reasonably follow China's recommendations for the control of COVID-19, including early testing, administering as many virus tests as possible, and early centralized isolation treatment. Compared with those of countries without any active policies, this strategy has better results. In China, the primary drug recommended for treatment is HCQ, along with other drugs such as α-interferon, lopinavir, ritonavir and umifenovir[15]. However, it should be noted that every patient is a unique individual, and personalized and precise treatment may be the most appropriate.

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Declaration of competing interests

The authors have no conflicts of interest or financial ties to disclose.

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Figure 1 Study Flow Diagram.

Table 1 Baseline Characteristics in the Full Study Cohort of 37 Hospitalized COVID-19 Patients.

Characteristic	Total	Experimental group	Control group	P
		HCQ or CQ	Routine treatment	
Group size	37	18	19	/
Male sex, No. (%)	16 (43.2)	5 (27.8)	11 (57.9)	0.065
Age, median (IQR), yr	60 (43.5-67.0)	55.5 (39.0-65.0)	65.0 (44.0-71.0)	0.328
Respiratory rate, median (IQR),/min	20.0 (19.0-20.0)	20.0 (19.0-20.0)	20.0 (18.0-20.0)	0.484
Symptoms, No. (%)				
Fever	23 (62.2)	12 (66.7)	11 (57.9)	0.582
Cough	27 (73.0)	12 (66.7)	15 (78.9)	0.401
Chest tightness	13 (35.1)	7 (38.9)	6 (31.6)	0.642
Muscle ache	5 (13.5)	2 (11.1)	3 (15.8)	0.677
Weakness	4 (10.8)	1 (5.6)	3 (15.8)	0.316
Diarrhea	3 (8.1)	1 (5.6)	2 (10.5)	0.580
Palpitation	3 (8.1)	2 (11.1)	1 (5.3)	0.515
Laboratory values, median (IQR)				
White cell count (10^{-9}/L)	5.6 (4.3-6.9)	5.4 (4.0-7.1)	5.7 (4.8-6.8)	0.515
Lymphocyte count (10^{-9}/L)	1.5 (1.0-1.9)	1.6 (0.9-2.0)	1.5 (1.1-1.7)	0.651
High-sensitivity C-reactive protein (mg/L)	8.2 (2.0-26.8)	11.4 (1.4-27.4)	7.7 (2.7-26.2)	0.609
Platelet count (10^{-9}/L)	222.0 (174.5-296.0)	216.0 (153.0-284.3)	240.0 (194.0-313.0)	0.250
Hemoglobin (g/L)	126.0 (114.5-136.0)	124.0 (114.8-135.5)	128.0 (113.0-145.0)	0.871
Serum albumin (g/L)	38.7 (35.5-41.1)	37.7 (34.8-39.9)	39.8 (36.5-41.5)	0.466
Serum creatinine (μmol/L)	65.0 (55.0-80.0)	63.0 (54.8-77.0)	68.0 (55.0-86.0)	0.762
Alanine aminotransferase ALT (U/L)	21.0 (15.0-44.5)	26.0 (13.0-52.5)	21.0 (15.0-42.0)	0.346
Aspartate aminotransferase AST (U/L)	23.0 (19.0-40.5)	27.5 (19.8-48.3)	21.0 (18.0-32.0)	0.358

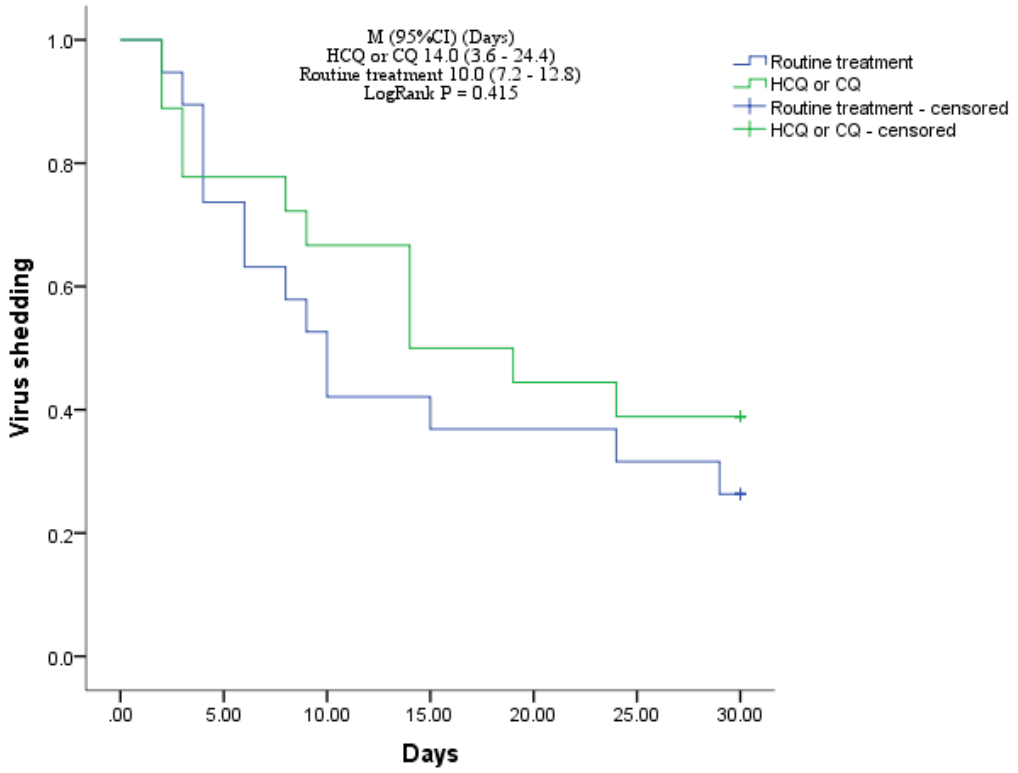
Drugs except HCQ or CQ, No. (%)				
Gamma globulin	3 (8.1)	2 (11.1)	1 (5.3)	0.515
Lopinavir-ritonavir	9 (24.3)	4 (22.2)	5 (26.3)	0.772
Zadaxin	15 (40.5)	10 (55.6)	5 (26.3)	0.070
Antibiotics	14 (37.8)	7 (38.9)	7 (36.8)	0.898
Hormones	3 (8.1)	2 (11.1)	1 (5.3)	0.515
Chronic conditions, No. (%)				
Hypertension	9 (24.3)	5 (27.8)	4 (21.1)	0.634
Diabetes	7 (18.9)	2 (11.1)	5 (26.3)	0.238
Coronary artery disease	3 (8.1)	2 (11.1)	1 (5.3)	0.515
Chronic respiratory diseases	3 (8.1)	1 (5.6)	2 (10.5)	0.580

*The values shown are based on available data. Laboratory values for white cell count, lymphocyte count, platelet count, high-sensitivity C-reactive protein, hemoglobin, serum albumin, serum creatinine, aspartate aminotransferase, and alanine aminotransferase were available for 18 patients in the experimental group and 40 patients in the control group. To convert the values for creatinine to milligrams per deciliter, divide by 88.4. IQR denotes interquartile range.

Table 2 Rate of patients with a negative viral load.

	Total	**Experimental group**	**Control group**	***P***
Negative viral load rate		**HCQ or CQ**	**Routine treatment**	
Total, No. (%)	25 (67.6)	11 (61.1)	14 (73.7)	0.414
Day 7, No. (%)	11 (29.7)	4 (22.2)	7 (36.8)	0.331
Day 14, No. (%)	20 (54.1)	9 (50.0)	11 (57.9)	0.630
Day 21, No. (%)	22 (59.5)	10 (55.6)	12 (63.2)	0.638
Day 28, No. (%)	24 (64.9)	11 (61.1)	13 (68.4)	0.642

Figure 2 Duration of viral shedding in the 37 hospitalized COVID-19 patients.


M (95%CI) (Days)
HCQ or CQ 14.0 (3.6 - 24.4)
Routine treatment 10.0 (7.2 - 12.8)
LogRank P = 0.415
Routine treatment
HCQ or CQ
Routine treatment - censored
HCQ or CQ - censored
Virus shedding
1.0
0.8
0.6
0.4
0.2
0.0
.00
5.00
10.00
15.00
20.00
25.00
30.00
Days