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Short Communication

# Experience of the use of hydroxychloroquine on patients with COVID-19: A perspective on viral load and cytokine kinetics



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## KEYWORDS

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Until now, there are no approved treatment against COVID-19. Hydroxychloroquine (HCQ) was hypothesized to be active against SARS-CoV2 via antiviral and anti-inflammatory effect; however, HCQ for COVID-19 in clinical use remained debating. In this preliminary report, we presented six patients with mild to moderate COVID-19. They were treated with HCQ for 14 days from the day of COVID-19 diagnosis. Serial viral load from respiratory specimens were performed every other day. Cytokine profile was checked before HCQ initiation and on the 14th day of HCQ treatment. All patients receiving HCQ completed 14-day course without complication. Among the six patients, the mean duration from symptom onset to last detectable viral

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load was  $34 \pm 12$  days, which was similar to those without specific treatment in previous reports. Low level of interferon-gamma was noted in all patients of different stage of infection and three patients had elevation of IL-17 level. Prolonged virus shedding is still observed regardless HCQ. The impact of HCQ on cytokine kinetics remained unclear; however, IL-17 could be an inflammatory marker for disease status monitor and a potential therapeutic target. Copyright © 2020, Formosan Medical Association. Published by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## Introduction

Coronavirus disease 2019 (COVID-19) has caused catastrophic pandemics since late 2019, with clinical features from asymptomatic infection to multiorgan failure owing to cytokine storm. Until now, there are no approved treatments against COVID-19, while many drugs have been hypothesized to be active via antiviral or anti-inflammatory effect.<sup>1</sup> Hydroxychloroquine (HCQ) was theoretically to be actively against SARS-CoV-2 via interfering with the glycosylation of ACE2, blocking virus/cell fusion and inhibiting lysosomal activity by increasing intracellular pH level, and have anti-inflammatory effect by inhibiting the production and release of TNF and IL-6.<sup>2</sup> Animal studies had demonstrated HCQ can efficiently inhibit SARS-CoV-2 infection in vitro, along with its anti-inflammatory function.<sup>3</sup> However, a previous retrospective study revealed that treatment with hydroxychloroquine, azithromycin, or both was not associated with significantly lower in-hospital mortality among COVID-19 patients.<sup>4</sup> Until now, efficacy of HCQ against SARS-CoV-2 infection remained debating. Besides, associated discussion of viral load kinetics after HCQ treatment is rare. Here we present six patients of mild to moderate COVID-19, with comprehensive virology and cytokine data.

## Material & methods

Between January 2020 and April 2020, there were 17 confirmed cases of COVID-19 hospitalized at National Taiwan University Hospital. They were confirmed through reverse transcription-polymerase chain reaction (RT-PCR) test of SARS-CoV-2 envelope (E), nucleocapsid (N), and RNA-dependent RNA polymerase (RdRp) gene. Six of them were prescribed with HCQ upon admission, with a loading dose of 400 mg twice daily for the first seven days, and followed by 200 mg twice daily for another seven days. Azithromycin was not given due to potential cardiac toxicity in the context of co-administration with HCQ.

During hospitalization, blood tests for hemogram, C-reactive protein, ferritin were checked twice per week, including the period of HCQ treatment. Cytokine profile was checked before HCQ initiation and on the 14th day of HCQ treatment. Chest radiograph were arranged once per week. Sputum, nasal swab and throat swab specimens were collected for examinations of SARS-CoV-2 RT-PCR every other day. Plasmid DNA containing the SARS-CoV-2 targeted

E gene, was used to construct the standard curve to estimate the SARS-CoV-2 viral load by real-time RT-PCR tests.

## Results

The demographic features of the patients are presented in the Table (See Table 1). Most of them were young female. All patients had mild respiratory symptoms before admission. None of them had respiratory distress. Initially, no leukocytosis or elevated C-reactive protein level was noted. At presentation, only one patient (Case 1) had fever ( $38.3\text{ }^{\circ}\text{C}$ ) with chills, and her chest radiography revealed increasing infiltration over bilateral lung fields. The other five patients had normal chest X-ray image upon admission. Fever and airway symptoms of all six patients resolved within a week after admission. No new subjective discomfort, including nausea, abdominal pain, diarrhea or palpitation, was complained during HCQ therapy. All patients were discharged without complication after three consecutive sets of negative RT-PCR results from sputum, nasal swab and throat swab specimens.

Virology data from upper and lower airway specimens are presented in Fig. 1. Those with more serious symptoms and admitted in the early stage of clinical symptoms tend to have higher viral loads of sputum specimens (Case 1–3,  $>10^5$  copies/mL), compared with those who were admitted in their late stage of infection (Case 4–6,  $<10^3$  copies/mL). Of Case 1, 2 and 3, viral load from upper airway specimens showed a decrease of 2 logs or more within seven days after starting HCQ treatment. Among the six patients, the mean duration from symptom onset to last detectable viral load was  $34 \pm 12$  days.

All six patients were found to have extremely low titers of interferon-gamma (IFN- $\gamma$ ) upon admission and on the 14th day of HCQ therapy. Most of the cytokines we checked upon admission were in low titers or undetectable; except for Case 5, who was admitted 14 days after initial symptom onset and was found to have a high IL-17 level. Three of the six patients were found to have mildly elevated IL-17 level after 14-day HCQ treatment. Mild elevated IL-6 level was observed in four of six patients upon their admission, and all patients had low level or undetectable IL-6 after HCQ treatment.

## Discussion

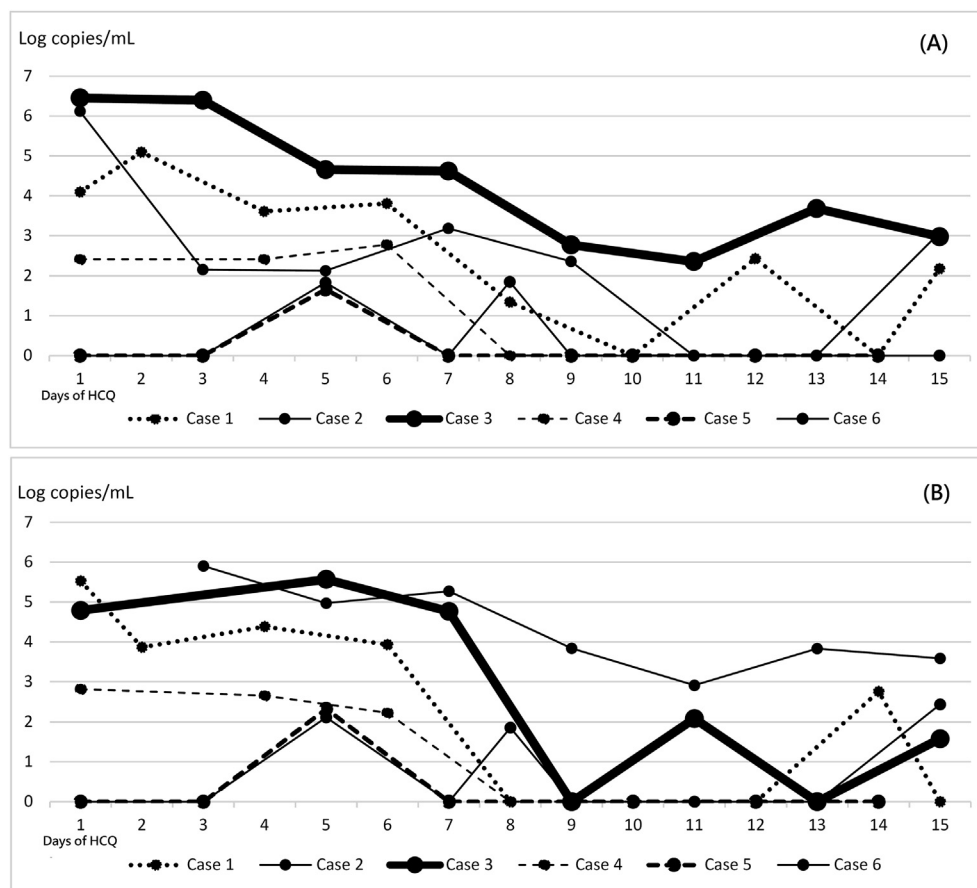
Until now, the efficacy of HCQ for treating COVID-19 remained controversial. A retrospective study by

**Table 1** Demographics, clinical features at admission, treatment, and outcomes of the patients.

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	
<b>Demographics</b>							
Age (years)	26	58	37	22	29	23	
Gender	Female	Male	Female	Female	Female	Female	
Comorbidities	Chronic urticaria	Hypertension			Allergic rhinitis	Allergic rhinitis	
<b>Clinical features on admission</b>							
Duration of symptoms before admission (days)	3	8	9	3	14	30	
Clinical features	Fever, chills, sore throat, malaise	sore throat, dry cough, cervical lymphadenopathy	dry cough, malaise, rhinorrhea, dyspnea	rhinorrhea	sore throat, malaise, productive cough, nausea, vomiting, diarrhea	loss of smell and taste, rhinorrhea	
Diagnosis	Pneumonia	URI	URI	URI	URI	URI	
<b>Symptoms and vital signs</b>							
Temperature (°C)	38.3	36.3	37	36.3	36.4	36	
Heart rate (/min)	86	66	94	78	78	84	
Respiratory rate (/min)	20	18	15	20	20	20	
Blood pressure (mmHg)	120/78	149/92	133/72	123/68	104/80	122/79	
O2 saturation in ambient air	100	99	98	100	100	100	
<b>Laboratory results</b>							
Hb (g/dL)	10.9	15	12.5	14	12.9	12	
WBC (cells/ $\mu$ L)	5230	3960	6150	2500	7950	6910	
Lym (%)	21.4	39.9	12	39.6	28.6	24.9	
Platelet (K/ $\mu$ L)	310	160	214	225	282	288	
CRP (mg/dL)	0.17	0.11	0.15	0.02	0.17	0.02	
ALT (U/L)	7	33	17	13	8	8	
LDH (U/L)	151	169	145	190	134	131	
CK (U/L)	31	162	45	47	29	58	
Ferritin (ng/mL)	5.85	459.44	42.58	72.74	138.11	3.76	
Viral load from throat swab specimens (copies/mL)	12,491	1,308,309	2,843,264	259	0	0	
Days of SARS-CoV-2 IgG detection after symptom onset (days)	17	14	28	NA	14 <sup>a</sup>	30 <sup>a</sup>	
<b>Cytokine profile (pg/mL)</b>							
IL-17	Day 0	0	0	0	114.74	0	
	Day 14	5.83	0	32.39	0.02	15.32	1.46
IFN- $\gamma$	Day 0	0	0	0	0	0	
	Day 14	0	0	0	0.21	0	0.06
TNF	Day 0	0	0	0	0	0.14	0
	Day 14	0	0	0	0	0	0
IL-10	Day 0	0.82	0.04	0.61	0	0.65	0
	Day 14	0.39	0.78	0.49	0.28	0.36	0.45
IL-6	Day 0	2.84	2.84	0	1.6	5.2	0
	Day 14	0	2.43	0.24	0.29	0	1.89
IL-4	Day 0	0.16	0	0	0	0	0
	Day 14	0	0.13	0	2.24	0	0
IL-2	Day 0	0	0	0	0.27	0.27	0
	Day 14	0	0.31	0	0.51	0	0
<b>Treatment and outcomes</b>							
Duration from symptom onset to last detectable viral load (days)	47	38	33	16	25	46	
Length of hospital stay (days)	52	35	28	22	20	24	
Outcomes	Survived	Survived	Survived	Survived	Survived	Survived	

**Abbreviation:** URI, upper respiratory infection; Hb, hemoglobulin; Lym, lymphocyte; CRP, C-Reactive protein; ALT, alanine amino-transferase; LDH, lactate dehydrogenase; CK, creatine kinase; IL, interleukin; IFN- $\gamma$ , interferon gamma; TNF, tumor necrosis factor.

<sup>a</sup> SARS-CoV-2 specific IgG could be detected from the serum sampled upon admission.



**Fig. 1** Viral load kinetics from respiratory specimens of throat swab (A) and sputum (B).

Rosenberg ES et al. revealed that treatment with hydroxychloroquine, azithromycin, or both was not associated with lower in-hospital mortality among COVID-19 patients. An open-label, randomized controlled trial of 150 patients with mild to moderate disease, also demonstrated that the use of HCQ did not show significant difference in negative conversion of SARS-CoV-2 by 28 days compared with standard of care.<sup>5</sup> Besides, a randomized trial by Boulware et al. also revealed HCQ did not prevent illness compatible with Covid-19 or confirmed infection when used as post-exposure prophylaxis within four days after exposure.<sup>6</sup> However, whether HCQ is able to prevent SARS-CoV-2 infection as pre-exposure prophylaxis remained uncertain.

Our study echoes the finding that the administration of HCQ is not associated with acceleration of virus clearance. Among our patients, three with initial higher viral load showed a decrease of 2 logs or more in their viral loads within seven days after starting HCQ treatment, which was similar to previous reports of patients without any antiviral or antiinflammation medications.<sup>7,8</sup>

Among our cases, three of them had elevation of IL-17 level, which was considered as an essential role in several inflammatory respiratory diseases. The reported cases demonstrated that the elevation of IL-17 was observed even in patients with only mild disease of COVID-19. Casillo et al. thus hypothesized IL-17 as a potential therapeutic

target.<sup>9</sup> Previous reports revealed that HCQ inhibits the differentiation of Th-17 cells and decreased the release of IL-17 from the experience of treating systemic lupus erythematosus.<sup>10</sup> Early administration of HCQ among our patients might inhibit the cytokine release and thus prevent hyperinflammation events, which potentially leads to deteriorating clinical condition. Nevertheless, the impact of HCQ on cytokine kinetics in COVID-19 patients still needs further studies.

In addition, low level of interferon-gamma was noted in our patients of different stages of infection, which implied atypical immune response against SARS-CoV-2 infection. Previous reports revealed that SARS-CoV-2 replicates stealthily in host cells without detectably triggering type I and III interferon, leading to high viral loads and impaired control for inflammation.<sup>11</sup> Our study demonstrated the immune response was impaired not only found in type I and III interferon, but also found in type II interferon.

Our case series demonstrated our experience of HCQ use in patients with mild to moderate COVID-19. Prolonged virus shedding is still observed regardless of HCQ. The impact of HCQ on cytokine kinetics remained unclear; however, IL-17 could be an inflammatory marker for disease status monitor and a potential therapeutic target. The impact on clinical course, viral shedding and cytokine kinetics still needs further investigation.

## Declaration of Competing Interest

none to declare.

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