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Retrospective Evaluation on the Efficacy of Lopinavir/Ritonavir and Chloroquine to Treat Nonsevere COVID-19 Patients

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Background: The effectiveness of lopinavir/ritonavir (LPV/r) and chloroquine treatment for COVID-19 has not been verified.

Methods: We conducted a retrospective study to summarize the clinical practices of nonsevere patients with COVID-19 receiving the standard care, LPV/r or chloroquine in Beijing Ditan Hospital from January 20 to March 26, 2020. The main outcome measurements include the changes of cycle threshold values of open reading frame 1 ab (ORF1ab) and nucleocapsid (N) genes by reverse transcriptase–polymerase chain reaction assay from day 1 to 7 after admission for patients receiving standard care or after treatment being initiated for patients receiving either LPV/r or chloroquine. The proportion of developing severe illness, fever duration and the time from symptom onset to chest computer tomography improvement, and negative conversion of nucleic acid were compared.

Results: Of the 129 patients included in the study, 59 received the standard care, 51 received LPV/r, and 19 received chloroquine. The demographics and baseline characteristics were comparable among the 3 groups. The median duration of fever, median time from symptom onset to chest computer tomography improvement, and negative conversion of the nucleic acid were similar among the 3 groups. The median increase in cycle threshold values of N and ORF1ab gene for patients receiving LPV/r or chloroquine or the standard care during the treatment course was 7.0 and 8.5, 8.0, and 7.6, 5.0, and 4.0, respectively. These figures were not found significantly different among the 3 groups.

Conclusions: Antiviral therapy using LPV/r or chloroquine seemed not to improve the prognosis or shorten the clinical course of COVID-19.

Key Words: COVID-19, efficacy, lopinavir/ritonavir, chloroquine

(*J Acquir Immune Defic Syndr* 2020;85:239–243)

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a newly emerging infectious disease caused by serious acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It first outbroke in Wuhan, China, and quickly spread across China as well as to other countries. As of May 10, 2020, more than 3 million confirmed cases of COVID-19 have been reported worldwide, including more than 27,000 deaths, and the overall fatality rate is about 7.0%.[1] Thus, there is a critical and urgent need for an effective treatment to cure severe patients and decrease the duration of virus carriage and limit transmission.

Beijing Ditan Hospital is one of the designated hospitals in Beijing, China, to manage patients with COVID-19. Because there was no proved treatment regimen for COVID-19, patients' treatment strategy was adapted according to the development and evolvement of the Chinese treatment guidelines for COVID-19. In the initial phase of the epidemic, there was no national treatment guideline available. Patients were given the standard care which was the symptomatic treatment strategy including oxygen and nutrition supplements, as well as the invasive ventilation and/or antibiotic treatment if indicated. The first draft of the Chinese treatment guideline for COVID-19 was released on January 16, 2020, and lopinavir/ritonavir (LPV/r) was introduced as one possible antiviral agent to treat COVID-19 because of its in vitro activity against the SARS-CoV[2,3] and some activity against Middle East respiratory syndrome coronavirus in animal studies.[4] After that, several revisions are being made based on the development of the epidemic and the relevant research updates. Although the evidence of the treatment of LPV/r to COVID-19 was not consistent,[5,6] clinicians at Beijing Ditan Hospital started to adopt LPV/r as the first-

Received for publication May 29, 2020; accepted July 6, 2020.

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The authors have no funding or conflicts of interest to disclose.

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line agent to treat patients with COVID-19 since early February 2020.

Chloroquine is another controversial drug for the treatment of the COVID-19. It is a drug mostly used for malaria and rheumatoid disease and was reported to have the ability to inhibit SARS-CoV from binding with angiotensin-converting enzyme 2 receptor and thus to stop the invasion.[7] A few nonrandomized studies has demonstrated a certain efficacy of chloroquine to treat COVID-19,[8–10] but the results seemed not convincing.[11] In late February, chloroquine was included in the newly released edition of the Chinese treatment guidelines for COVID-19[12] and doctors started to prescribe it based on their decisions.

Up to now, the effectiveness of LPV/r or chloroquine for COVID-19 remains contested. Therefore, we retrospectively summarized our clinical practices among nonsevere COVID-19 patients with the stand of care and with these 2 drugs aiming at providing a data basis to explore the next step in the treatment of COVID-19.

MATERIALS AND METHODS

Study Design and Patients

We conducted a retrospective study on the patients with COVID-19 hospitalized in Beijing Ditan Hospital. All patients were laboratory confirmed for the infection of SARS-CoV-2 using the qualitative reverse transcriptase–polymerase chain reaction (RT-PCR) method (Shanghai BioGerm Medical Technology Co., Ltd., Shanghai, China). The positive result was determined when the cycle threshold (Ct) values of both target genes were ≤38, and negative when they were both >38. If only one of the target genes had a Ct value ≤38 and the other >38, it was interpreted as a single-gene positive. Single-gene positive was also considered as positive in our study. The inclusion criteria of our study were patients (1) aged 18–65 years, (2) were classified as nonsevere cases on admission, (3) received treatment at least 72 hours, and (4) were admitted into the hospital after less than 7 days from symptom onset.

Treatment approach: (1) the standard care; (2) LPV/r: oral treatment, 500 mg twice a day for 7 days; and (3) chloroquine: oral treatment, 500 mg, twice a day for 7 days.

Data Collection and Definitions

We collected the patients' information from their electronic medical records which were set up after admission, including basic demographics (sex, age, and chronic disease), the time of diagnosis, antiviral therapy, the Ct values of the reference genes on d1 and d7 after admission or antiviral treatment, and clinical outcomes (the proportion of severe cases development, and the time from symptom onset to fever recovery, chest computer tomography (CT) improvement, and negative nucleic acid conversion) for each patient. Severe cases were defined as arterial oxygen partial pressure/oxygen concentration ≤300 mm Hg.[12] The time to negative nucleic acid of the respiratory specimen was defined as the time from symptom onset to nucleic acid tested negative for 2 consecutive times at 24 hours intervals.[12] The observation endpoint was April 30. The changes of Ct values of the reference gene from admission to day 7, the proportion of severe cases, and the time from illness onset to fever recovery, chest CT improvement, and negative nucleic acid conversion were used as measurements to evaluate the therapeutic effect.

Statistical Analysis

Continuous variables were expressed as medians (interquartile ranges), as appropriate based on the results of the Kolmogorov–Smirnov test for normal distribution. Categorical variables were presented as counts and percentages. The Wilcoxon rank-sum test was used for the continuous variables. The χ^2 test and Fisher exact test were used for the categorical variables, as appropriate. To compare the therapeutic effect of the antiviral treatment group and the control group, univariable logistic regression models were used. All analyses were conducted with SPSS 16.0 (IBM, Armonk, NY). P values <0.05 were considered statistically significant. $P1$ and OR1, $P2$ and OR2 refer to the P value and odds ratio (OR) between the control group and LPV/r or between the control group and the chloroquine group, respectively.

Ethnic Statement

The study was approved by the Ethics Committee of Beijing Ditan Hospital (Institutional Review Board (018) -01, 2020). Written informed consent was obtained from all patients.

RESULTS

Demographic and Baseline Characteristics

Among the 129 patients who met the inclusion criteria, 59 (45.7%) received the standard care, 51 (39.5%) received the treatment of LPV/r, and 19 (14.7%) received the treatment of chloroquine. The median time from symptom onset to treatment initiation was 5 (3–7) days. Seventy (54.3%) patients were male and distributed similarly among the 3 groups (30 vs 30 vs 10, $P = 0.696$). The median age was 33 (24–44) years and were comparable [30 (23–45) vs 33 (27–41) vs 32 (22–50), $P = 0.916$]. Eleven (8.5%) patients had pre-existing chronic diseases: 4 in the standard care group, 4 in the LPV/r group, and 3 in the chloroquine group. We also collected the Ct values of the N gene and ORF1ab gene from 25 patients in the LPV/r and the chloroquine treatment group on the first day and seventh day after the antiviral treatment and from 19 patients in the standard care group on the first day and seventh day after the hospital administration. On the first day, the median Ct values of N gene was 30 (25–34) in the standard care group, 27.7 (28.3–30.6) in the LPV/r group, and 29.5 (24.9–31.6) in the chloroquine group ($P = 0.478$). The median Ct values of ORF1ab gene was 30.0 (25.0–35.0) in the standard care group, 27.0 (23.9–30.8) in the LVP/r group, and 29.3 (24.9–35.9) in the chloroquine group ($P = 0.573$). There were no significant differences in demographic and baseline characteristics among the 3 groups (Table 1).

Treatment Outcomes

Overall, all 83 febrile patients' temperature returned to normal with a median time of 8 (5–10) days. Up to April 30, 2020, most patients showed chest CT improvement (82.2%, 106/129) and converted to negative on RT-PCR test (83.7%, 108/129) with a median time of 15.0 (13.0–19.3) and 23 (15–33.8) days, respectively.

As is shown in Table 2, the median duration of fever was similar among 3 treatment groups: 8 (6.0–11.0) days in the standard care group, 7.5 (4.8–9.3) days in the LPV/r group, and 6.0 (4.0–9.0) days in the chloroquine group. The median time from symptom onset to chest CT improvement was also comparable in 3 groups with 15.5 (11.0–20.8) days in the standard care group, 13.0 (15.0–19.0) days in the LPV/r group, and 14.0 (12.0–20.0) days in the chloroquine group. The median duration from symptom onset to the conversion to negative of RT-PCR test was also comparable in 3 groups with 21.0 (15.0–28.8) days in the standard care group, 23.0 (17.0–35.5) days in the LPV/r group, and 16.0 (14.0–41.0) days in the chloroquine group. During hospitalization, 6 (4.7%) patients developed serious illness: 2 from the standard care group, 3 from the LPV/r group, and 1 from the chloroquine group.

Overall, the median increase in Ct values of N gene and ORF1ab gene was 9.0 (3.9–13.3) and 8.9 (3.0–13.4) from first to seventh day, respectively. In details, the median increase in Ct values of N gene was 5.0 (2.0–11) in the standard care group, 7.0 (5.0–12.5) in the LPV/r group, and 8.0 (6.6–20.8) in the chloroquine group (the standard care group vs the LPV/r group: OR = 0.92, 95% confidence interval (CI): 0.800 to 1.055, $P1$ = 0.228; the standard care group vs chloroquine group: OR2 = 0.90, 95% CI: 0.798 to 1.002, $P2$ = 0.055). For the ORF lab gene, the median increase in Ct values was 4.0 (2.0–8.0) in the standard care group, 8.5 (5.4–13.5) in the LPV/r group, and 7.6 (4.5–21.0) in the chloroquine group (the standard care group vs the LPV/r group: OR = 0.92, 95% CI: 0.808 to 1.039, $P1$ = 0.172; the standard care group vs chloroquine group: OR2 = 0.92, 95% CI: 0.838 to 1.009, $P2$ = 0.077). As of April 30, 2020, 108 (83.7%) patients were discharged, and no death occurred.

DISCUSSION

In this study, compared with the control group, no evidence was found that treatment of LPV/r or chloroquine could shorten the clinical course of disease, including the fever duration and the time from symptoms onset to improvement of pneumonia and negative nucleic acid conversion in the respiratory specimen. There were no deaths in this study group, but 4.7% of the patients developed hypoxemia and developed severe illness during treatment. Antiviral therapy with LPV/r or chloroquine did not stop the disease progression into severe cases. We also compared the Ct value of viral nucleic acid in respiratory tract samples of each group on the first day and the seventh day of hospitalization or treatment and found no significant difference in the Ct value changes between the control group and the antiviral drug groups, but the viral N gene nucleic acid Ct value of the chloroquine group was significantly higher than that of the control group (P = 0.055).

COVID-19 is a new infectious disease caused by SARS-CoV-2 infection. At present, there are no effective antiviral drugs or vaccines for treatment. According to previous antiviral treatment experience of other types of coronaviruses, the diagnosis and treatment guideline of COVID-19 issued by the National Health Committee has recommended the repositioning use of old drugs such as LPV/r and chloroquine to treat COVID-19, both of which have been used for specific diseases for decades, thus entitling us to a very rich and comprehensive experience in application and propounding grasp of their side effects. Under these circumstances, no serious adverse cardiac events or any withdrawal because of serious side effects were observed in this study.

Lopinavir is an antiviral drug widely used in the treatment of HIV. Lopinavir is an effective component of the drug, whereas ritonavir can inhibit CYP3A-mediated metabolism of lopinavir, thus increasing the concentration of lopinavir in plasma. The use of LPV/r in the treatment of coronavirus has been reported for a long time. For example, Chu et al[2] analyzed the antiviral effect of lopinavir combined with ribavirin in 41 patients with SARS in Hong Kong and found that the total mortality (2.3% vs 15.6%) and intubation rate (0% vs 11.0%) were significantly better than those of ribavirin alone. In addition, in a nonhuman primate model, subjects who were treated with LPV/r or interferon-β-1b for MERS-CoV had a better prognosis than those who did not receive treatment.[4] However, in this study, the LPV/r treatment did not shorten the time of fever recovery and pneumonia improvement, nor did it accelerated the clearance of the virus, indicating that there was no benefit from the antiviral treatment. Previously, in a randomized, controlled, open-label trial of hospitalized adult patients with severe

TABLE 1. The Demographics and Baseline Characteristics of 129 Patients

Characteristics	Total (n = 129)	Control Group (n = 59)	Lopinavir/Ritonavir Group (n = 51)	Chloroquine Group (n = 19)	*P*
Male, n (%)	70 (54.3)	30 (50.8)	30 (58.8)	10 (52.6)	0.696
Age, median (range), yrs	33 (24–44)	30 (23–45)	33 (27–41)	32 (22–50)	0.916
Comorbidities	11 (8.5)	4 (6.8)	4 (7.8)	3 (15.8)	0.515
Time from symptom onset to treatment initiation, median (range), d	5 (3–7)	—	5 (3–7)	3 (3–7)	
Ct value of N gene, median (range)	29.0 (24.5–31.8)	30 (25–34)	27.7 (28.3–30.6)	29.5 (24.9–31.6)	0.478
Ct value of ORF1ab gene, median (range)	28.6 (24.3–34.3)	30 (25–35)	27 (23.9–30.8)	29.3 (24.9–35.9)	0.573

TABLE 2. Comparison of Clinical Outcomes Between the Control Group and the Treatment Group

Clinical Outcomes	Total (n = 129)	Control Group (n = 59)	Lopinavir/Ritonavir Group (n = 51)	Chloroquine Group (n = 19)	Control vs Lopinavir/Ritonavir		Control vs Chloroquine	
					*P*1	OR1 (95% CI)	*P*2	OR2 (95% CI)
Fever duration, median (range), d	8 (5–10), n = 89	8 (6–11), n = 44	7.5 (4.8–9.3), n = 38	6 (4–9), n = 7	0.146	0.910 (0.802 to 1.033)	0.690	0.958 (0.778 to 1.181)
Time from illness onset to, median (range), d								
Chest CT improvement	15 (13–19.3), n = 114	15.5 (11–20.8), n = 52	13 (15–19), n = 47	14 (12–20), n = 15	0.949	1.003 (0.92 to 1.084)	0.770	0.984 (0.886 to 1.093)
Negative nucleic acid conversion	23 (15–33.8), n = 119	21 (15–28.8), n = 59	23 (17–35.5), n = 45	16 (14–41), n = 15	0.285	1.018 (0.985 to 1.051)	0.839	1.004 (0.963–1.047)
Severe cases, n (%)	6 (4.7)	2 (3.4)	3 (5.9)	1 (5.3)	0.882	1.163 (0.158 to 8.568)	0.714	1.583 (0.136 to 18.5)
Changes of Ct value from day 1 to day 7, median (range)								
N gene	9.0 (3.9–13.3), n = 44	5 (2–11), n = 19	7 (5.0–12.5), n = 13	8.0 (6.6–20.8), n = 12	0.228	0.918 (0.800 to 1.055)	0.055	0.895 (0.798 to 1.002)
ORF1ab gene	8.9 (3.0–13.4), n = 44	4 (2–8), n = 19	8.5 (5.4–13.5), n = 13	7.6 (4.5–21), n = 12	0.172	0.916 (0.808 to 1.039)	0.077	0.920 (0.838 to 1.009)

SARS-CoV-2 infection conducted by Professor Cao Bin's team, where 99 patients were given LPV/r therapy in addition to standard care and 100 control cases were given standard care alone, it was concluded that no benefit was observed with LPV/r treatment beyond standard care, which was consistent with our findings.[6]

The study of chloroquine in the treatment of coronavirus was first conducted during the SARS epidemic from 2002 to 2003, but the mechanism is not clear. Based on the preliminary study of SARS-CoV-2, it is suggested that the virus enters the cell by binding to the angiotensin-converting enzyme 2 receptor, and chloroquine may prevent the virus from binding to the receptor by inhibiting terminal glycosylation.[13] At the same time, chloroquine can enter the endosome and lysosome, resulting in the increase of intracellular pH, which then leads to the degradation of defective proteins essential for virus infection, replication, and reproduction.[14,15] Since the outbreak of COVID-19, some clinical studies have also shown that chloroquine treatment could shorten the clinical course.[10,16] In addition, in vitro experiments also proved that chloroquine and hydroxychloroquine had inhibitory effect on SARS-CoV-2.[17,18] However, in this study, compared with the control group, the chloroquine treatment showed no advantage in improving symptoms, accelerating negative nucleic acid conversion or reducing the incidence of severe disease. Despite that, the increase in the Ct values of nucleic acid in respiratory specimen from d1 to d7 after taking chloroquine was more significant than the control group. Therefore, the treatment course, dosage, and effectiveness of chloroquine for COVID-19 still require higher-quality clinical trials.

There are some shortcomings in this study. First, this study was retrospective and there was no strict study design as to the antiviral treatment, which will inevitably lead to a certain statistical deviation. Second, patients started antiviral therapy at different stage of the clinical course, which may have an influence on the observation of the therapeutic effect of the drugs. Finally, the number of patients enrolled in the group is relatively small.

In conclusion, treatment of nonsevere COVID-19 patients with LPV/r or chloroquine seemed not to accelerate the remission of symptoms, improve the prognosis, or shorten the clinical course. A large randomized controlled trial is needed to provide solid evidence.

ACKNOWLEDGMENTS

The authors thank the authors for their timely and helpful responses to our information requests. The authors appreciated Dr. Liming Wang for her technical support on critical review and editing of the article.

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